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SOME ASPECTS OF ECOLOGY AND ONTOGENY IN THE FISHES AND AMPHIBIANS[1]

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Although long tradition combines the study of amphibians and reptiles into one field, these two vertebrate classes are usually treated entirely separately, in actual practice, even when both are discussed in the same paper. Comparative papers on amphibians and reptiles are rare, except for those that inquire into basic evolutionary problems from the paleontological viewpoint. Typically, we specialize in details and overlook the broadly comparative problems that cross departmental and taxonomic lines. Perhaps discussion of some of the opportunities offered by a different and less familiar combination of taxonomic groups can make the advantages of interdepartmental study more tangible. The fishes and amphibians invite such comparative study, for these two classes present some important common problems in life history and evolutionary biology.

Effective comparison of fish and amphibian life histories is somewhat hampered by numerical and ecological differences. The Recent amphibians are remnants of a once very large and complex group, but the Recent fishes represent a continuing great diversification of a long-dominant group. Furthermore, the fishes are aquatic throughout life, but most amphibians have, in addition, both the advantages and the problems of terrestrial life during part of their ontogeny. Within the limits imposed by such differences, however, much can be learned from comparative studies on life history patterns and on developmental characters, in relation to the varied ecological situations that these animals face.

We commonly base ecological and distributional studies on the adult stage only, and overlook the possibility that the relative success or failure of a species may be determined largely by events that affect its early developmental stages, rather than its more conspicuous adult. The critical importance of survival rate in early stages is recognized most clearly by fishery biologists, who are concerned with size of population of commercial

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species and with the contribution that each successive year-class makes to that population. The fishes and amphibians are excellent materials for comparative study of environmental conditions that influence the place of a particular species in the fauna through their effects on critical stages in its ontogeny. Ecological life history studies on these animals could be considered from several different viewpoints, for ecology is an integrative science that draws its data from many fields. The present paper reviews some of the evolutionary background of life history study, and then discusses some ecological and developmental problems in the fishes and amphibians, centered chiefly around the varying importance of the larval stage in dispersal and gene exchange.

THE EVOLUTIONARY BASIS OF LIFE HISTORY CHARACTERS

Evolution involves complex interactions between environmental selection on the one hand and genetic potentialities and limitations on the other. These controls do not restrict their operation to the adult characters, but affect every aspect of the life cycle.

Paleontology can reveal very little of the actual course of life history evolution, but comparative study of many living groups with diverse relationships gives much indirect evidence of evolutionary trends in developmental characters, breeding habits, physiological characters, general behavior, etc. It is easier to recognize that these are valid topics for evolutionary study if we keep in mind the well-established principles that the entire ontogeny of an individual is a unit governed by a single genetic system, and that variation and change can modify either the whole ontogeny or any part of it.

The first amphibians were doubtless little more than a population of aberrant crossopterygian fishes. For some time the prospective new group and its close relatives probably retained a rather similar genetic makeup in most respects, and continued to face similar ecological situations and to share common life history characters. Further evolution widened the gap between them, not only in adult characters but in the entire ontogeny. Meanwhile, the precursors of the main lines of teleost evolution also became established. Their subsequent evolution has been very elaborate in all aspects of morphology and development, and is still inadequately understood. The taxonomy and evolution of the fishes and amphibians are highly complex subjects, and need evidence from all possible sources, including developmental characters.

THE ROLE OF THE LARVAL STAGE IN DISPERSAL AND GENE EXCHANGE

The importance of the larval stage in the general biology of the species and in the evolution of groups is both complex and variable. For example, in many invertebrates, especially marine groups, the dispersal of the species depends on pelagic larvae. In some other groups, notably some of the insects, the larva is a sedentary feeding and growing stage, and the adult is restricted to a brief, mobile, reproductive period at the end of a

long larval life. In some cases the larva confers no obvious advantage on the species, and its continuance in the ontogeny is perhaps little more than an ancient genetic tradition.

Even in a relatively restricted group, such as the lower vertebrates, the actual function of the larva in the life of the species varies widely. In the non-parasitic brook lampreys, for example, the larva is a prolonged feeding and growing stage, and the adult has become reduced to a terminal, non-feeding, reproductive stage. In many of the non-migratory marine fishes distribution depends largely on pelagic eggs and larvae, for the adults settle in limited territories and do not wander far after maturity. The fishes and amphibians offer many opportunities for comparative studies on the role of the larval stage in maintaining populations or in promoting gene exchange between populations.

Freshwater fishes

In freshwater fishes, geographic spread is guided largely by the pattern of lakes and stream systems. All developmental stages are aquatic and have access to essentially the same migration routes. In general, it is likely that the adult is usually the chief agent in dispersal and gene exchange, except perhaps in large lakes where pelagic larvae would foster mixing of populations, and in crowded streams in which accidental hybridization may occur.

A substantial amount of the known natural hybridization of freshwater fishes, especially in rivers and streams, may result not from direct interspecific matings but accidentally from the drift of freshly spawned eggs and sperm, especially where ecological modifications have restricted the available spawning sites to small overcrowded areas that are used simultaneously by two or more species (work by Carl L. Hubbs and collaborators; e.g., Hubbs and Miller, 1953). Although these situations perhaps usually result from man-made ecological changes, they could certainly also stem from natural physiographic disturbances. Hubbs (in several papers) has suggested that the apparent greater frequency of natural hybridization of freshwater fishes than of marine forms is probably due in part to the greater opportunities for chance mixing of eggs and sperm of related species in freshwater streams, and to the less stable ecological conditions in fresh waters.

The known eggs of most freshwater fishes are demersal. They are either attached to some supporting object, or are heavy enough to sink to the bottom, or are buried in sand or gravel. Eggs of some freshwater forms, although technically demersal, are only slightly heavier than water and are non-adhesive. These eggs are subject to action by currents, which may roll them about or keep them more or less suspended, and may even carry them a considerable distance before hatching takes place. Examples include some of the cyprinids, and the striped bass, *Roccus saxatilis*. The striped bass is a diadromous fish, but it is appropriate to mention its freshwater eggs here. A few freshwater fishes are known to have true pelagic

eggs, free-floating at or near the surface. These include some of the gouramies, the eggs of which can float at the surface independent of support by the bubble nest. This has long been known. An account by Ryder (1886) was apparently overlooked by Eigenmann, who stated in his widely quoted paper (1909) that he knew of no freshwater pelagic eggs. At least one freshwater clupeoid, *Amphiodon alosoides*, has pelagic eggs (Sprules, 1949), and additional examples can be expected. In general, however, true pelagic eggs are uncommon in freshwater fishes, and are not known to provide conspicuous opportunities to spread the species into new territory. The occurrence of pelagic eggs in certain freshwater clupeoids is probably chiefly of genetic rather than ecological significance, i.e., retention of the type of egg that characterizes most of the marine members of the group.

Freshwater fishes display a rather limited range of known larval specializations. This apparent limitation in variability is probably partly explainable on ecological grounds. The amphibians must bridge the gap between larva and adult with both a morphological and an ecological metamorphosis, but in the freshwater fishes the larva and the adult occupy essentially the same kinds of environments. Hence, in the latter group there has been much less pressure on the larva to "make good" in a sharply different ecological niche, as a critical link in the success or failure of the species. The fishes as a whole do have ample genetic capacity for larval variability, as is well shown by the great range of developmental specializations in the marine fishes.

Amphibians

Amphibians that are completely aquatic in all developmental stages have patterns of dispersal and opportunities for gene exchange similar to those of freshwater fishes. Speciation and distribution in these animals are influenced by the geography and ecology of drainage systems, and by similar factors that reflect restricted environment and limited avenues for movement. In these fully aquatic forms the larva is perhaps neither more nor less important than the adult as an agent in distribution and population stability.

By far the commonest and best-known life cycle in the amphibians comprises aquatic egg and larval stages and terrestrial adult stage. Within this general framework the details of ecology, breeding habits, and developmental characters vary widely. The role of the larva in extending the geographic or genetic importance of a particular species varies with the taxonomic group and with the general ecological circumstances. Larvae that live in ponds or temporary pools are closely confined, and hence wandering and gene exchange in these species are necessarily functions of the post-metamorphic juveniles or of the adults. Climatic and taxonomic variables and physiological requirements influence the relative importance of larva and adult in the dispersal of different species that breed along interconnecting waterways rather than in isolated pools. In a favorable climate, an adaptable species may do considerable overland wandering in

the adult stage, resulting in significant mixing of local colonies. A form less capable of overland travel, or living in a climate less favorable for it, would disperse mainly along the home stream system. Here, perhaps larva and adult would be equally effective in dispersal. If there have been significant differences of this sort in mode of dispersal, their influence on evolution might be discernible in present-day patterns of distribution and speciation. A careful study of North American amphibians from this viewpoint might be profitable.

The greater evolutionary success of the frogs than of the salamanders (comparing them at the ordinal level) is certainly related in part to the much greater capacity of the frogs for genetic variability in developmental characters. Tadpoles probably do not often lead their species into extensive new areas, but larval evolution and adaptation have kept pace successfully with adult wanderings into new areas and adaptations to peculiar habitats. The distributional significance of the tadpole is therefore chiefly indirect. A non-migratory species that has a confined larval stage cannot live in a particular area unless both its larva and its adult can live there. Highly successful evolution in developmental characters has allowed the frogs to succeed in many situations that would be closed to them if the group as a whole were too inflexible in developmental requirements (Orton, 1953).

Tadpoles that are highly specialized for peculiar ecological conditions are known from many parts of the world. There is extensive adaptive radiation in larval characters not only in the frogs as a whole, but also within many of the families and even within some of the larger genera. Equally important is the extensive convergent evolution through which larvae of unrelated forms have secondarily evolved very similar adaptations. For example, many kinds of tadpoles are modified for life in swift mountain brooks. The more extreme kinds have a flattened head and body, long flat snout, greatly enlarged mouthparts, thick heavy tail muscles, and reduced tail fins. Convergence toward this morphological and ecological type is conspicuously duplicated by unrelated larvae in at least six families, but the precise details (especially of the mouthparts) differ widely and in general retain basic characters of the particular group from which the specialized larva is derived. Many examples of gradations in complexity between this extreme specialization and the more ordinary tadpole format are known. Stream adaptation in tadpoles is a very attractive ecological and evolutionary problem, and greatly needs further study.

Larval adaptation helps a species to live under special conditions, but it might also ultimately restrict the species to those conditions. If extreme specialization in tadpoles is genetically irreversible, it is conceivable that many groups of frogs might eventually specialize themselves into extinction through modification of their larvae into extreme end-forms, unable to cope with subsequent ecological changes. Here, larval ecology involves the basic genetic question of reversibility of evolution, another very attractive problem that is beyond the scope of this paper.

One of the most successful ontogenetic specializations in the frogs—direct development—has nearly eliminated the larva as a recognizable

stage. Amphibians that have direct development skip the free-living aquatic tadpole and are terrestrial at all stages. The factors that modify their distribution approach those of any group of strictly terrestrial animals. Much has been published on the developmental morphology and general ecology of amphibians with direct development (e.g., Lutz, 1947, 1948; Orton, 1949, 1951), but little on population studies that relate this modified ontogeny to the over-all economy of the particular species. In recent work on a leptodactylid genus, Syrrhophus, (reported at the Pullman AAAS meeting, June, 1954) Jameson found the adult frogs to have small home ranges, but the newly hatched terrestrial young wander much more widely and are evidently the stage responsible for most of the dispersal and gene exchange in these frogs.

The factors that govern direct development in amphibians provide an interesting field for study and speculation. Perhaps the most obvious and most-often stated point is that direct development frees the species from dependence on open water for its eggs and larvae, and the species can then live successfully in areas that lack suitable aquatic habitats. Direct development is, however, not a single culminating achievement in the frogs, but is an independent specialization in many unrelated lines. In some regions (some of the West Indies, some Pacific islands) amphibians with direct development are the dominant, or the only, amphibians. But direct development is by no means confined to species that live in areas which do not provide normal habitats for an aquatic tadpole. A species that develops in terrestrial sites is freed from larval competition and thus gains a selective advantage even in regions that are well provided with swamps or streams. For example, the very large and complex genus Eleutherodactylus and certain other closely related genera form an important part of the frog fauna throughout the neotropics, and so far as their life histories are known they all have direct development. That larval competition can be a real factor in the success of a species is obvious to anyone who has watched the dense tadpole populations in small ponds. The successful establishment and spread of colonies of the accidentally-introduced *Eleutherodactylus ricordi planirostris* in Florida, studied by Goin (1947), is a striking example of the ability of a frog with direct development to compete in a region that is saturated with a very rich native fauna of frogs with aquatic larvae.

The taxonomy of early developmental characters has been studied much more extensively in the amphibians than in the freshwater fishes. This would be surprising enough if the two groups were of nearly the same size and importance, but the better-studied amphibians are a much smaller group and have a minimum economic importance. The rich opportunities for correlated developmental and taxonomic studies on freshwater fishes have been much neglected. This peculiar hiatus in knowledge stands out especially sharply to those who enter ichthyology after previous training and experience in herpetology. It illustrates the need for greater awareness of problems and successful methods in other fields in addition to one's own speciality.

Diadromous fishes

In the freshwater fishes and the amphibians, the role of the larva in dispersal and gene exchange is often minor or indirect. But in the diadromous fishes, and especially in the marine fishes, the situation changes and the larva assumes a greater direct significance as a factor in population dynamics.

Migratory species in many groups of fishes regularly spend different stages of their life cycle in waters that contrast in salinity or in other physico-chemical factors. An ecological classification of all these fishes would involve virtually all conceivable intermediate patterns of migration and combinations of habitats. In order to simplify general reference to such fishes, Myers (1949) proposed the new term "diadromous" to apply to any or all fishes that undergo a breeding migration into waters of different salinity.

The best-known diadromous fishes are two groups that display extremes of difference in migratory pattern: the salmonids with marine adults that enter freshwater streams to spawn and have large eggs and a large, yolk-bearing larva ("alevin"); and the freshwater eels that go to the sea to spawn and have pelagic eggs and a peculiar leptocephalus larva.

The significance of the larva in the distribution of diadromous fishes varies, depending on the direction and ecological details of its migration, and on the characters of its taxonomic group. The marine salmonids that hatch in fresh water and mature in the sea typically return to their home stream for spawning. Downstream migration of the larvae is rather strictly channeled, and any effective range extension would be done primarily by the adults, through errors in navigation on the return spawning run. These conditions minimize effective mixing of populations, and favor local differentiation of genetically isolated stocks. In other groups of fishes that enter fresh water to spawn, the migration and mixing of stocks in different developmental stages may be more complicated and less conducive to population stability. For example, the striped bass runs a variable distance upriver into fresh water to spawn, but its semi-pelagic eggs may be carried downstream for a considerable distance, and may enter brackish water or even sea water before hatching (Raney, 1952). This fish is now under intensive study along the Atlantic, and in the San Francisco Bay region (where it has been introduced), to determine the extent of the coastwise migrations of adults and young, and the possible importance of migration in maintaining or mixing populations.

Problems of population dispersal are somewhat different in fishes that have the reverse pattern of migration, i.e., spawning at sea and maturing in fresh water. If sea spawning occurs in estuaries or close inshore, there would be limited opportunity for gene exchange by means of drifting eggs or larvae, and in such cases localized differentiation associated with river systems might be the rule. If spawning takes place far offshore, the genetic as well as the geographic situation could be quite different. If all the freshwater eels of the western north Atlantic drainage go to the same

general area in the sea to spawn, with no segregation in time or space, there is theoretically opportunity for a very thorough mixing of each generation that could keep the entire eel population relatively homogeneous, with no tendency to split into localized races. Johannes Schmidt found such uniformity to characterize the European eel, but this problem has apparently never been investigated in the American eel. A long larval migration through the open sea to reach the adult habitat puts a heavier burden on the eel larva than does the relatively simple downstream migration of the salmonid larva. There are still many unanswered questions on this aspect of the life history of our freshwater eel.

Marine fishes

In the marine fishes the larval stage attains its greatest importance, among the vertebrates, as an agent in dispersal and gene exchange. Two major factors in marine ecology that influence distribution are: the huge continuous space that is theoretically available in the sea, and the widespread occurrence of free-floating pelagic eggs and larvae, which comprise part of the vast community of the marine plankton. The dispersal of pelagic early stages of marine animals has been compared aptly with the wind-borne dispersal of the pollen and seeds of terrestrial plants (Garstang, cited by Hardy, 1954).

The adventures of the pelagic egg and larva are very important to successful completion of the life cycle of the individual, in addition to their potential importance in extending the geographic range of the species. Plankton life is highly competitive. It is heavily dominated by invertebrates, and fish eggs and larvae comprise only a very small part of it. In some groups of fishes both the egg and the larva are pelagic (e.g., sardines and mackerels); in others, the egg is demersal but the larva is pelagic (e.g., blennies and gobies). The enormous production and enormous waste of pelagic eggs and larvae have been commented on by many writers. Norman estimated that a 21½ pound cod produces 6½ million small pelagic eggs; in contrast, a 20 pound salmon produces only about 17,000 large demersal eggs. Johannes Schmidt estimated a Mola to contain 300 million eggs. The plankton drifts with the ocean currents, and is affected by many additional factors in the physical environment. These conditions vary greatly from time to time, and in some years oceanographic events may be very unfavorable for the survival of certain species, through mass failure of the larvae to reach areas or conditions that are needed in their later development. As a consequence, the relative success of year-classes may differ within very wide limits, especially in species that produce very high numbers of eggs. Ahlstrom (1954) pointed out that: "The initial strength of a year-class is dependent on the amount of spawning; its subsequent strength, on survival after spawning. In actual numbers of individuals, a year-class at its inception is many times as large as the total adult population.... the history of each year-class is one of rapid decrease in numbers." He estimated the

survival rate in the 1950 and 1951 year-classes of the Pacific sardine as one larva surviving to the 21 mm. stage per 1000 eggs spawned. Sette (1943) estimated survival in the 1932 year-class of the Atlantic mackerel as between one and ten larvae surviving to the 50 mm. stage per million eggs spawned. He considered this an abnormally low survival rate for this species, resulting from unusually adverse conditions in that season.

Various authors, interested in the effectiveness of pelagic young as distributing agents, have investigated the duration of these stages in relation to the direction and distance they may be carried before leaving the plankton. Regan (1916) suggested that coastal species that occur in different areas widely separated by open sea probably have a long pelagic stage; in general, coastal forms that have a short pelagic stage would not be dispersed as widely and are likely to have more restricted ranges. Larval dispersal is, of course, not the sole determinant of range size or range continuity. Other factors, such as length of residence of the group in the area, also modify distribution. Bonnet (1939) studied the drift of pelagic stages of the Atlantic cod, which spawns in winter at very low temperatures. He summarized that eggs spawned off Massachusetts at 3°C hatch 23 days later, and the larvae remain drifting in the plankton for seven days, before settling to the bottom, making a total time of about 30 days in the plankton. The estimated drift of four miles per day would take them about 120 miles, and would land the settling larvae on the edge of Georges Bank, a favorable bottom area for young cod.

Clark Hubbs (1953) discussed the extensive distribution of Labrisomus, a genus of clinid blennies that is represented in the eastern Pacific and on both the American and African shores of the Atlantic. It has the longest pelagic larval stage of any clinid known to him, and he suggested that it probably reached Africa by trans-Atlantic migration in the larval stage. Williams (1954) treated the bearing of larval distribution on a complicated subspecies problem in Gibbonsia, another clinid genus in the eastern Pacific. Here both allopatric and sympatric subspecies had been described. He noted that the allopatric forms differ in meristic characters, which are usually fixed in early developmental stages, and concluded that these forms probably constitute definite genetic populations. The described sympatric forms, however, differ only in proportions and other characters that can be modified by the environment very late in development. He postulated that these described sympatric forms are not separate genetic stocks but are derived from a single ecologically-widespread population that becomes thoroughly mixed during the pelagic larval stage, and that the proportional and other differences develop after the individual larvae have settled in a variety of different environments.

These examples indicate but a few of the kinds of problems in the field of larval ecology of marine fishes. The value of such study is manifold, for it contributes toward better understanding of the systematics and evolution of the fishes, as well as toward practical knowledge of biological factors that control productivity of an important part of man's food supply.

SUMMARY AND CONCLUSIONS

In the freshwater fishes and the amphibians, the role of the larva in distribution and gene exchange is often minor or indirect; but in the diadromous fishes, and especially in the marine fishes, the larva assumes a greater direct significance as a factor in dispersal and population dynamics. The sharpest contrasts are between the frogs and the marine fishes. In the frogs the outstanding distributional significance of the larva lies in its genetic variability, which permits adaptation to a very wide range of ecological conditions. This high degree of larval adaptability has helped the frogs to distribute themselves very widely, and it has certainly been an important factor in the greater evolutionary success of the frogs compared with the salamanders. In the marine fishes the larva commonly has a very direct role in dispersal of the species, through a more or less prolonged stay in the drifting plankton.

The fishes and amphibians share many basic problems that can be studied effectively by comparison and by interchange of ideas. Progress in the study of either of these groups can suggest the means for substantial advance in the other, not only in ecological topics but in other problems concerning ontogeny. For example: (1) Patterns of larval evolution in the frogs are virtually a blueprint to guide the study of developmental characters in the fishes. Although the problems are much more complex in the fishes, they appear to involve essentially the same principles. (2) The year-class concept, which is so important in population studies on marine fishes, could be applied effectively on amphibians and reptiles. It would give both a broader understanding of population ecology in these animals, and a greater realization of the importance of the whole ontogeny as the unit in the study of species problems.

Early developmental stages of the fishes and amphibians show many modifications that are more or less obviously adaptive to the environment of the particular species, and these characters offer many interesting ecological problems. In addition to ecological study, however, there is need for extensive investigation of the taxonomic and phylogenetic stability of these characters, and the nature of their evolutionary trends.

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ASPECTS OF OSMOTIC REGULATION IN CRABS SHOWING THE TERRESTRIAL HABIT

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INTRODUCTION

The adaptive value of osmotic regulation among aquatic animals especially where extreme osmotic gradients are sustained, for example, among fresh water and brine lake dwellers, is an obvious one. However, there is evidence that such a physiological process also appears in forms showing a tendency to the terrestrial habit. The question then arises whether or not osmotic regulation is also adaptive here. It is true that osmotic regulation is a step toward homeostasis, which offers a degree of independence from the environment, but it is not necessary *a priori* that an animal must regulate osmotically in order to live on land, for it is conceivable that animals which possess highly impermeable integuments and high tolerance to water loss could spend considerable time out of water without harm. Or they could seek environments of high humidity and thus reduce the hazard of evaporation. The Onychophora show tremendous tolerance to water loss, and dwell in humid situations. (Manton and Ramsay, 1937; Prosser et al, 1950.) It would therefore seem unnecessary for them to be able to regulate against a major stress.

The question then may be posed: How general is the occurrence of osmotic regulation among terrestrial forms? In this paper terrestrial and semi-terrestrial crabs will be considered, and some factors in the mechanism which may have permitted the process of osmotic regulation *per se* to allow the direct invasion of land from the sea will be sought.

Jones (1941) pointed out that strong osmotic regulation accompanies the tendency to the terrestrial habit of life in crabs, and in particular that some degree of hypo-regulation (maintenance of blood osmotic pressure below that of medium) is present in animals capable of living on land. He also emphasized that strong hypo-regulators are always strong hyper-regulators, that is, regulators in dilute sea water. Pearse (1932) has shown that crabs with terrestrial behavior tend to have blood which is normally hypotonic to the sea. Since many such animals frequently become immersed in sea water, they are, in effect, hypo-osmotic regulators. The present investigation will add further evidence that regulation characterizes such species.

The major evolutionary invasions of land by animals are not believed to have come directly from the sea. Rather, the evidence indicates that re-

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curring conditions of aridity during the Silurian and Devonian periods desiccated bodies of fresh water and encouraged adaptations for terrestrial life among the arthropods, molluscs and vertebrates (Lull, 1917). Thus, these forms were already strong hyper-osmotic regulators and their ancestors had probably attained the fresh water habit by degrees, having first left the sea by way of brackish water. In a sense, then, hyper-regulation has furnished a pathway to life on land.

However, some invasions of land have come directly from the sea. This is evident among the land crabs which are still bound to the sea by their reproductive habits. The evolution of osmotic regulation in these animals is not so easy to explain.

Jones (1941) proposed that hypo-regulation in land crabs would be adaptive by regulating against the branchial fluids made concentrated by evaporation and thus preventing the blood from reaching a harmfully high osmotic pressure.

Jones' suggestion concerning the adaptiveness of hypo-regulation will be examined by direct experiments; then the ability of the same species to take up water against a gradient will be shown. Finally, the adaptations of the coconut or robber-crab *Birgus latro* for land life will be demonstrated.

MATERIALS AND METHODS

The new information reported concerns the following species. The shore crab *Pachygrapsus crassipes* Randall was collected at Ballona Creek and Flat Rock Point, California. *Birgus latro* Linnaeus, the coconut or robber-crab, was collected on the island of Guam in the Pacific Ocean and flown to Los Angeles where it was studied in the laboratory for several months. Birgus was also studied in the field at Bikini atoll, along with *Grapsus grapsus* (Linnaeus), a tropical semi-terrestrial, rockdwelling crab, *Ocypode sp*, a tropical semi-terrestrial burrowing crab and the land hermit-crab *Coenobita sp*.

Blood osmotic pressure measurements were made by the melting point method (Gross, 1954) and expressed in terms of per cent sea water, 3.48 per cent salt being considered 100 per cent sea water). Salt or water exchange between organism and known volume of medium was followed by measuring electrical conductivity with a 1000 cycle bridge. The cell used required only a 2 ml. sample which was not expended. This method was introduced by Pantin (1931). Respiratory measurements were made by means of the Scholander-Wennesland respirometer.

RESULTS

Osmotic Regulation in Terrestrial and Semi-Terrestrial Crabs. Pachygrapsus immersed in concentrated or dilute sea water maintains a blood melting point intermediate between that of the medium and its normal value

in sea water after 72 hours immersion. Per cent regulation is determined by dividing the maintained osmotic gradient by the gradient which would be maintained if no change occurred in the blood concentration. Typical animals score 33 per cent on this scale in 150 per cent sea water and better than 60 per cent in 50 per cent sea water. Among the several score determinations made, it was observed that the scatter of values approximates that observed by Jones (1941). It should be pointed out that Pachygrapsus struggles violently in a hypertonic medium.

The osmotic behavior of the land crab, Birgus, was demonstrated by a similar experiment, but this undertaking was complicated somewhat because this animal will drown if indefinitely immersed. It was necessary therefore, to allow the experimental crabs to rise slightly out of the water to permit respiration. This, of course, would not permit the maximum stress which a given medium could impose on an animal, but it did allow much of the body surfaces to be covered at all times. It was found that Birgus under these laboratory conditions was regulating 69 per cent in 25 per cent sea water after 24 hours and 27 per cent perfect in 137 per cent sea water after 48 hours.

The osmotic behavior of Birgus in dilute sea water was also studied in the field at Bikini atoll, along with the osmotic behavior of three other land crabs, *Coenobita sp*, *Grapsus grapsus* and *Ocypode sp*[2]. All of these crabs will drown if completely immersed. Experiments in the field show that Birgus, Grapsus and Ocypode drown in running sea water within 24 hours. Coenobita removed from its snail shell expires after 36 hours. Thus the same procedure and limitations mentioned above apply here. Animals were partially immersed in about 5 times their respective volumes of solution and salinity changes of the medium were measured by means of a conductivity bridge. Assuming that solute space is equal to 50 per cent of the body volume it was found that Birgus was regulating about 60 per cent in a certain fresh water (ship's drinking water) after 72 hours; Coenobita about 50 per cent in 25 per cent sea water after 33 hours, and Ocypode perfectly in 35 per cent sea water after 33 hours.

Although the degree of avoidance of immersion by standing high out of the water varies (Ocypode exhibits this most), it nevertheless seems clear that land and semi-terrestrial crabs are, in general, strong hyper-osmotic regulators.

It was demonstrated above that Birgus can regulate in concentrated sea water, but no further direct experiments have been conducted to demonstrate the general tendency for hypo-regulation among land crabs. However, data in Table 1 show that blood hypotonic to the sea is generally present in crabs showing some degree of terrestrial behavior. As pointed out above, this would effectively be hypo-regulation since these forms frequently enter the sea.

[2]All field observations at Bikini were kindly made by Professor Theodore Holmes Bullock, July, 1948.

It can be said, then, that both terrestrial and semi-terrestrial crabs can regulate osmotically, that there is a correlation between terrestrial life and hypo-regulation and that hypo-regulation in crabs is found only where hyper-regulation is also found.

Evaluation of Branchial Fluid Theory of Jones: Determination of Branchial Fluid Volume—Pachygrapsus freshly removed from normal sea water, ranging in size from 15 grams to 35 grams, were placed in desiccators in which there was no air current, at a temperature of 16° C, and were observed

TABLE 1

MOST CRABS OF TERRESTRIAL AND SEMI-TERRESTRIAL HABIT HAVE DILUTE BLOOD

Species	Typical environment	Blood concentration (% sea water*)	
Birgus latro	True land habit; found great distances from seashore	64	(2)
		90	(1)
Coenobita clypeatus	True land habit; even found in desert-like situations	102.5	(5)
		64	(2)
Coenobita cavipes		64	(2)
Coenobita sp.		102	(1)
Gecarcinus lateralis	Burrows among vegetation in sandy areas near sea or distantly from sea	81	(5)
Cardisoma guanhumi	Burrows among mangroves and other vegetation, particularly along estuaries and marshes	81.5	(5)
Sesarma haematocheir	Burrows in banks in proximity of fresh water	68	(4)
Ocypode albicans	Burrows in sand near sea; enters sea often	83.5	(5)
Grapsus grapsus	Wave-swept rocky shores; enters sea frequently	94	(5)
Uca crenulata	Burrows in sand at edge of seashore; frequently enters water	85	(3)
Pachygrapsus crassipes	Wave-swept rocky shores; spends more time in water than out	85	(3)
		108	(1)
		107	(5)

1-present investigation
2-Harms (1932)
3-Jones (1941)
4-Pearse (1931)
5-Pearse (1932)

*Relative to sea water of respective locale.

for viability and weight loss as a function of time. Such animals usually survived at least 24 hours; most survived for 48 hours and some remained alive for as long as five days. The maximum body weight loss during such periods was about 11 per cent.

In order to determine what part of the weight loss can be accounted for by evaporation of sea water from the gill chamber and also to determine what salinity changes are caused by evaporation in the branchial fluid, it was necessary to determine the approximate volume of the branchial fluid present in crabs in air, but recently removed from an aqueous medium. This was

accomplished by flushing out the gill chamber with distilled water and measuring the electrolytes in the flushings.

Holes about 1 mm in diameter were drilled into the gill chamber of crabs which were then placed in a dry container for about one hour to facilitate blood clotting. After such a period the animals were returned to normal sea water for at least 24 hours. The external surface of such an animal was rinsed with distilled water to wash off any salt, care being taken to prevent mixture with the gill fluid. The blunted needle of a syringe was placed in the drilled holes and distilled water was forced into the cavity, the jet being directed to cover the entire internal surface. Approximately 10 ml. of water for each side was found sufficient to flush the salts out of the chamber. The flushings were then caught and the electrical resistance was measured by a conductivity bridge. Knowing the volume of the flushings then, and assuming that the gill fluid was 100 per cent sea water, the volume of that fluid can be calculated from the electrical resistance interpreted in terms of per cent sea water.

Determinations made in such a manner on 20 animals show considerable variation ranging from 0.65 per cent body weight to 3.3 per cent body weight for the combined fluid of both branchial cavities. The mean was 1.7 per cent. Because of the osmotic stress imposed on the branchial tissues by distilled water, there is probably some loss of salt from the body fluids during the few seconds of flushing, thus increasing the calculated value for the volume of branchial fluid. Nevertheless the volume of fluid carried in both branchial cavities of Pachygrapsus is small, rarely exceeding 3 per cent of the body volume.

Salinity Changes in the Gill Chamber During Desiccation—If salinity in the gill chamber could now be estimated, the maximum transfer of salt from the chamber to the blood could be calculated, when the animal is exposed to desiccation. If water is evaporated and there is no absorption of salts from the hypertonic solution by the branchial tissues, then the total electrolytes in the gill cavity should measure the same, before and after desiccation. If, however, there is absorption there will be less salt in the chamber after desiccation.

The branchial fluid volume in a group of Pachygrapsus recently removed from normal sea water was determined by the above described method. After the determination, the crabs were returned to sea for 24 hours, in order to assure replacement of sea water in the gill chamber. The animals were then blotted, weighed and placed in a desiccator until they had lost substantial weight. Their external surfaces were then rinsed carefully with distilled water to remove salts and again the gill chamber was flushed out, and electrolytes measured in the flushings. Results shown in Table 2 reveal that salts in the gill chamber average 17 per cent of the original, after desiccation. This means that (a) salts are absorbed by the branchial tissues or (b) branchial fluid is lost by draining out or being removed or (c) both occur.

The above procedure was therefore repeated on five specimens of Pachygrapsus, which instead of being placed in desiccators were put in contain-

ers which were maintained humid by wet paper towels located where they could not be reached by the crabs. Results on such controls kept in humid containers for at least as long as the above crabs were desiccated, show there is an average salt content after the experiment of 91.2 per cent. There were no overlapping values between the two groups "t" evaluation showing the difference to be valid at the <0.1 per cent level of probability, and it can be said that even if there is some loss of the branchial fluid by drainage

TABLE 2

SALT ABSORBED FROM FLUIDS IN BRANCHIAL CAVITY OF PACHYGRAPSUS

Specimen No.	Weight change during experiment (g)	(% Body Wt.)	Volume of gill fluid before desiccation (ml)	Salt in gill chamber after desiccation (% original present)	Salt change equivalent to m equiv. NaCl
Experiments (In desiccated situation)					
1	−1.71	6.1	0.76	13	−0.41
2	−1.36	4.9	0.92	0	−0.65
3	−0.20	0.79	0.79	54	−0.22
4	−0.30	1.1	0.81	9	−0.46
5	−0.63	2.8	0.55	24	−0.25
6	−2.18	9.2	0.31	10	−0.18
7	−2.59	7.0	0.86	12	−0.48
8	−2.72	8.4	0.65	17	−0.34
Mean	−1.46		0.71	17	−0.37
Controls (In humid situation)					
9	+0.76*		0.86	100	0
10	−0.03		0.72	67	−0.15
11	−0.01		0.65	123	+0.09
12	+0.33*		0.64	111	+0.04
13	−0.05		0.44	55	−0.12
Mean	+0.20		0.66	91	− .03

*These animals were weighed only before the gill chamber was flushed. Since they were immersed in sea water after this to return the branchial fluid to normal, the weight gained merely indicates that the animals picked up more water during the second immersion, e.g., by drinking.

during desiccation, the reduced values for salt content in the gill chamber must mainly be accounted for by absorption of the salts by the branchial tissues.

The question then arises as to the role of the absorbed salts in elevating the osmotic pressure of the blood when the animal is exposed to an arid environment. The concentration of the blood of living crabs was therefore determined by melting point before and after desiccation, and the relative effect of branchial cavity salts in elevating the concentration of the blood was calculated, assuming an extreme value of three per cent body weight as the amount of fluid originally found in the chamber. Table 3 shows that the absorbed salts could have only a small role in determining the increased osmotic pressure of the body fluids compared with the total change. This amounts to a maximum of a 6 per cent increase over the original concentra-

tion, assuming that the branchial fluid and the mobile water together sum 50 per cent of the body weight. Assuming that ion- and osmo-regulating tissues line the branchial cavity and with the knowledge that Pachygrapsus can maintain its blood hypotonic to concentrated sea water, it is obvious there must be a mechanism at least partially inhibiting the absorption of salts from the branchial fluids when they become concentrated by evaporation. Because of the comparatively exposed nature of the gills, it seems that evaporation should occur from them more rapidly than from internal

TABLE 3

THE RELATIVE ROLE OF BRANCHIAL SALTS IN ELEVATING THE BLOOD CONCENTRATION OF DESICCATED PACHYGRAPSUS

Specimen	Weight loss % body weight	Observed change in concentration of blood. (% sea water)	Maximum increase in blood* Concentration caused by absorption of branchial fluid salts (% sea water)
1	10.5	+44	+6
2	6.6	+23	+6
3	9.1	+30	+6
4	10.8	+32	+6
5	7.8	+26	+6

*Assuming that the branchial chamber fluid = 3% body wt., and that the animals mobile water + gill chamber fluid = 50% body wt.

structures through the chitinous exo-skeleton, or even through the protected gut openings, and therefore the maximum effect of the branchial fluid salts in elevating the body fluid concentration would be felt early. Table 2 shows that small weight losses parallel large salt losses from the gill chamber. In view of the high blood concentrations which are tolerated by Pachygrapsus both in natural and laboratory situations (table 3, 4) it seems improbable that a 6 per cent increase in blood salinity would cause effective harm during the early stages of desiccation. Thus hypo-osmotic regulation which is so common in crabs showing terrestrial habits would not be effectively adaptive by inhibiting absorption of branchial fluid salts into the body fluids, at least in the case of Pachygrapsus. The validity of this conclusion does not depend on the accuracy of the calculated values for solute space and branchial fluid volumes. Even if the effect of branchial fluid salts were doubled an early 12 per cent increase in the osmotic pressure of the blood would have little influence on the crabs' behavior. In fact, animals taken in nature in terrestrial situations show such high blood concentrations (table 4).

Hypo-osmotic regulation then either has other adaptive values or is the superficial manifestation of a more subtle phenomenon. Now it can be observed in tables 2 and 3 that in general, considerably more weight was lost during the period of desiccation than could be accounted for by complete evaporation of the branchial fluid. This means that water is lost through other pathways, or that water is released into the branchial chamber as it

TABLE 4

BLOOD CONCENTRATIONS OF PACHYGRAPSUS IN NATURAL SITUATIONS

o.p.-osmotic pressure: (% S.W.)-equivalent to per cent of normal sea water.

Specimen number	Osmotic situation	o.p. of medium (% S.W.)	o.p. of blood (% S.W.)
1	turbulent water	100	114
2	(open coast)	100	100
3		100	107
4	tide pools	101	108
5		103	110
6		104	108
7	dry rocks		135
8			119
9			101
10	burrows (mud flats)		105
11			104
12			108

evaporates. Again it can be seen in table 2 that salt in the gill chamber becomes depleted by an average of 83 per cent of its original quantity when the animal is desiccated. This means that if fluid is secreted into the branchial chamber, its electrolyte content is low.

Secretion of Branchial Fluid—If Pachygrapsus were able to keep its gill chamber moist in an arid situation by secretion, then it might be that the gills would lose less weight by evaporation proportionately than the rest of the body. Five animals were therefore desiccated to a moderate loss (less than 10 per cent body weight). The gills were then removed and weighed and the weight loss of the gills was estimated from measurements on gills of 10 animals freshly removed from sea water. Results show that while the desiccated crabs lost less than 10 per cent of their original body weight, their gills weighed an average of 32 per cent less than those of the control animals which were freshly removed from sea water. Thus in every case it was indicated that the gills lost more proportionately than the rest of the body. Since salt has been shown above to be absorbed with the gradient as it is established but is not completely absorbed, either the residual salts cannot get through the membranes which have become dried, or water is secreted into the gill chambers. Such water would maintain an equilibrium and thus prevent a salt gradient from being increased. The pronounced desiccated condition of the gills does not support this view, but there are other membranes in the gill chamber and these have not been studied.

Effects of Desiccation on the Respiratory Rate—It has been suggested by Jones (1941) that drying of respiratory membranes in Pachygrapsus limits its terrestrial life. An attempt was therefore made to demonstrate the effect of desiccation on the respiratory rate of Pachygrapsus and whether an effect, if present, is caused by lack of fluid in the gill chamber. An animal was removed from normal sea water and placed immediately in a respirometer

where its rate of oxygen consumption was measured. It was then placed in a desiccator until a significant weight loss was observed. After this period the oxygen consumption of the crab was again determined. After such a period the gill chamber in some animals was made wet with normal sea water. This was accomplished by a syringe through holes previously drilled into the chamber. After this operation the oxygen consumption of the crab was again determined. Other crabs were further exposed to evaporation and repeated measurements were made of their rates of oxygen consumption. Figure 1 illustrates the typical behavior of animals so treated. The oxygen

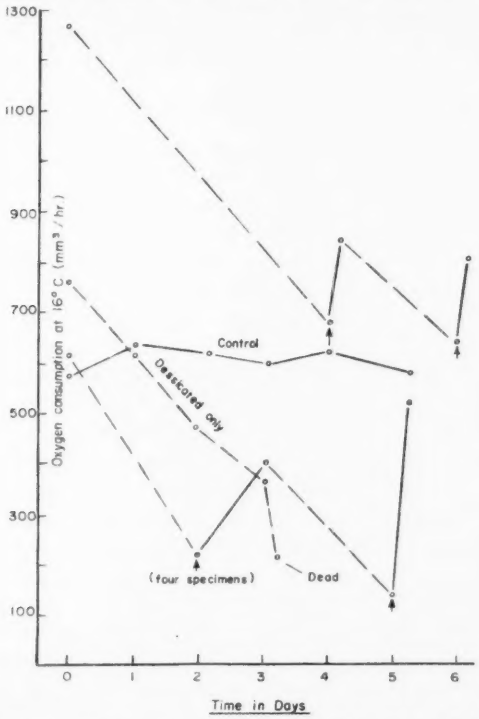

FIGURE 1. Pachygrapsus consumes less oxygen after a period of desiccation. Such a condition is partially ameliorated by artificially wetting the gill chamber. Broken line = period of desiccation; arrow = point where sea water is introduced into the gill chamber; control = respiration in air of animal freshly removed from sea water. Control animal kept in sea water between determinations.

consumption of a crab decreases as it is exposed to evaporation. This decline appears with one day's desiccation. However, immediately before death, respirometer readings indicate that significant gaseous exchange is still occurring (200 mm³/hr), but at the same time the gills appear rather dry. The tissues lining the gill chamber do not appear dry and may therefore at least be assisting in the gaseous exchange.

When the branchial chambers of a desiccated animal are moistened, the respiratory rate usually increases but does not generally return to its pre-

desiccation rate. Assuming that moistening of the gills returns the initial transport efficiency to the respiratory membranes, it seems that the total metabolism of an animal may be lowered independently of the oxygen that can be transported, and does not return immediately to normal when the supply of oxygen reaching its tissues again increases. So it appears that desiccation depresses an animal's metabolic rate more than by simply drying some of the membranes. Aerial life in Pachygrapsus certainly must be limited because the respiratory membranes lose efficiency with desiccation. But it seems doubtful that moderate desiccation is particularly harmful, through any lowering of respiration since animals often survived after depression to 200 mm^3/hr, at least for many hours. Jones' proposal is therefore probably correct but only for long maintained drying.

ACTIVE UPTAKE OF WATER BY PACHYGRAPSUS

The experiments described above indicate adaptations in Pachygrapsus for semi-terrestrial life. They can respire effectively out of water for extended periods and can tolerate considerable drop in metabolism when the efficiency of their respiratory membranes is reduced by desiccation. The behavior of Pachygrapsus returned to water after a prolonged period in the air was therefore investigated. Animals which had been exposed to evaporation producing considerable weight losses were returned to measured volumes of sea water. The weight changes in the crabs were observed and the salt changes in their respective media were measured by a conductivity bridge. Results show a rapid uptake of water, and in some cases, a loss of salt to the medium. It was therefore investigated whether there is an active uptake of water and/or excretion of salts under such conditions.

Crabs which were exposed to evaporation producing measured weight losses (approximately 10 per cent body weight) were immersed individually in volumes of 140 per cent sea water which were equal to twice the respective volumes of the animals. The concentration 140 per cent sea water was calculated from table 3 to be hypertonic to the blood of the animals in all cases. The weight changes of the animal and the salinity changes of the medium were then measured as a function of time, adequate precautions being taken to guard against salinity changes caused by evaporation. If the medium is taken in "as is," the electrical conductivity of the medium will not change; if only water is taken in the conductivity will increase; if more salt is taken in than is already present in the medium, per unit volume of solution, then the conductivity will decrease. The relative quantities of salt and water uptake can be expressed by the ratio: calculated medium change – observed medium change ÷ calculated medium change—where the calculated change is the expected salinity change in the medium assuming that the weight change by the animal is caused by pure water. The observed change is that determined by measurement of electrical conductivity.

Results in figure 2 illustrate that water is taken up against a gradient, considering the concentrations of the blood and the medium. At first, water is taken in along with a relatively large amount of salts which enter with

the gradient. As time goes on, however, water continues to be absorbed with it; this may be the result of a reduced salt gradient. In no case was there a net loss of salt to the medium from that originally present in the blood. This is to be expected since the crab has actually gained no salt by being desiccated. There is evidence, however, that salt which is taken in at the same time with water gradually becomes excluded with time. In such cases the medium demonstrates an actual net gain in electrolytes, per unit volume of solution. This suggests that water may be taken up by

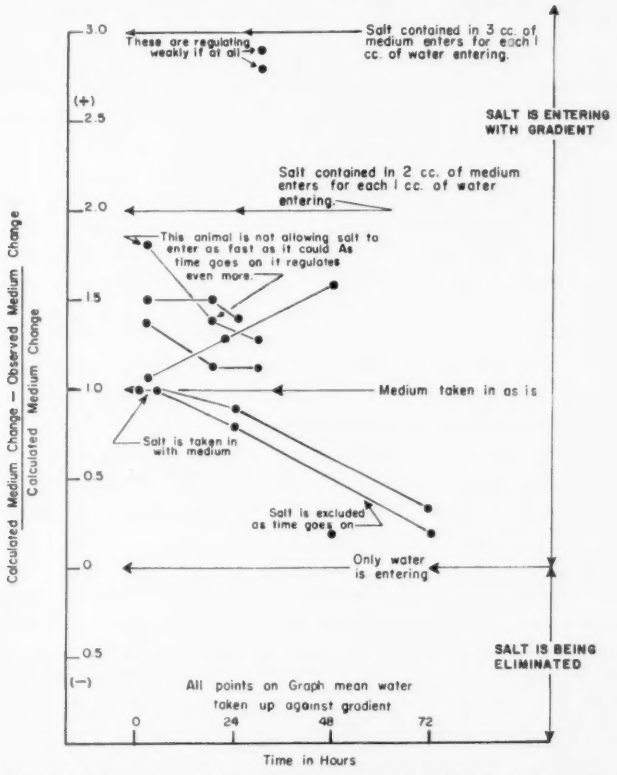
3.0
These are regulating weakly if at all
Salt contained in 3 cc. of medium enters for each 1 cc. of water entering.
(+)
2.5
SALT IS ENTERING WITH GRADIENT
Salt contained in 2 cc. of medium enters for each 1 cc. of water entering.
2.0
This animal is not allowing salt to enter as fast as it could As time goes on it regulates even more.
1.5
1.0
Medium taken in as is
Salt is taken in with medium
0.5
Salt is excluded as time goes on
0
Only water is entering
0.5
SALT IS BEING ELIMINATED
(−)
All points on Graph mean water taken up against gradient
0
24
48
72
Time in Hours
Calculated Medium Change − Observed Medium Change / Calculated Medium Change

FIGURE 2. Slightly desiccated Pachygrapsus put in 140 per cent sea water gains weight. Water is actively taken up and salt actively excluded. All points are indicated. Solid lines represent history of individual specimens.

drinking. The observation that there is no immediate increase in the salinity of the medium when a desiccated crab is immersed in 140 per cent sea water, but that the increase occurs only gradually with time supports this contention.

It may be argued that the water uptake is merely the refilling of the branchial cavity, and it is true that the total measured uptake will include such water.

However, as shown above, the volume of branchial fluid is about three per cent at maximum and these crabs lost approximately 10 per cent by evaporation.

Absorption of water against a gradient can be accomplished by Pachygrapsus. Such a phenomenon as absorbing water actively then may be part of the mechanism of hypo-osmotic regulation, but more, it may be in itself an important factor which has encouraged the direct invasion of land by marine crabs.

ADAPTATIONS TO TERRESTRIAL LIFE BY THE LAND CRAB *BIRGUS LATRO*

The ability to regulate osmotically is present in the coconut or robber crab Birgus although as an adult this species makes little use of such a process for the function of sustaining an osmotic gradient between its body fluids and the external environment. This anomuran crab as an adult has made the full step to life on land, but has failed to divorce itself completely from the sea where it must return to reproduce. Birgus then may be said to represent an evolutionary trap for it cannot again become completely aquatic because immersion in water eventually results in drowning. The respiratory membranes, namely, the gills and branchial tufts are adapted only for aerial respiration.

Even in an aerial environment, the osmotic properties of the body fluids are likely to be subject to change depending on the stresses imposed. Therefore the effects on the body fluids under certain conditions that Birgus might meet in nature were investigated.

The coconut crab leads a terrestrial existence in regions where both fresh and sea water are available (Reyne, 1939). It is known that the animal must return to the sea to breed, but its behavior relative to fresh water is not clearly described.

Five specimens of Birgus maintained in Los Angeles were placed where they could choose between an aquatic and an aerial habitat. Two of these were situated where only normal sea water was available. Another two were placed where only fresh water was available. The fifth specimen was given the choice of both fresh water and normal sea water. Since these two media are present in the coastal parts of the natural environment of this species, the last of the above laboratory conditions simulates best its osmotic situation in nature. The crabs were exposed to these situations for several weeks (table 5) during which time weight changes were frequently determined. No food was offered during these periods. When an animal was finally removed from one of the above conditions, the concentration of its blood was determined by melting point method. The concentration of the available water was then changed, and after another period of exposure, the blood concentration was determined again.

Results of these experiments (table 5) established that the blood concentration of Birgus is influenced by the salinity of the available water. The blood of a specimen exposed only to fresh water became dilute while the blood of a specimen exposed only to sea water became more concen-

trated. Yet the blood concentration of the crab which could reach both types of water was intermediate in concentration between those of the other crabs. Osmotic pressure of the body fluids as a function of time was not studied in the last experiments. However, results suggest that when both fresh water and sea water are available, osmotic equilibrium probably occurs within one week (table 5).

The weight data for the above experiments were not accurate enough for quantitative analysis, but they did show significant fluctuation, indicating that the crabs were taking on and losing water at regular intervals. Lister

TABLE 5

THE BLOOD CONCENTRATION OF BIRGUS FOLLOWING CONSUMPTION OF FRESH WATER OR NORMAL SEA WATER

o.p.-osmotic pressure; (% S.W.)-equivalent to per cent of normal sea water; spec. no.-specimen number; avail.-available; expos.-exposure; enviro-environment; F.W.-fresh water; S.W. normal sea water.

A Spec. No.	B Water avail.	C Days exposed	D o.p. of blood after exposure (% S.W.)	E water changed to:	days in new enviro.	o.p. of blood after new expos. (% S.W.)
1	F.W.	66	69	F.W. and S.W.	15	88
2	F.W.	52	64			
3	S.W.	78	118	F.W. and S.W.	8	89
4	S.W.	52	123	F.W. and S.W.	15	85
5	F.W. and S.W.	52*	92	F.W.	9	74

*exposed to same environment for several days before experiment.

(1888), in describing the drinking of sea water by Birgus, relates that the liquid is picked up by the chelipeds, and is transferred to the maxillipeds which in turn pass it on to the other mouth parts. This investigation confirms Lister's observation for the drinking of both fresh water and sea water. In addition, however, it was observed that occasionally the last pair of thoracic appendages, which is usually tucked into the branchial cavity, comes forward during the above action to take water from the maxillipeds, thence passing it on into the branchial chamber. This procedure would be an excellent means of moistening the respiratory membranes. A similar behavior has been described for the isopod *Ligia baudiniana* which is able to moisten its gills by dipping the uropodial spines into water, drawing it onto the respiratory membranes by capillary action (Barnes, 1932).

It is obvious that the tissues of Birgus exhibit tolerance to a wide range of blood concentrations. Specimens with extreme body fluid concentrations

equivalent to 64 per cent, and 123 per cent sea water appeared no less active than the crab with the intermediate concentration equivalent to 92 per cent sea water.

The blood concentration of Birgus therefore varies according to the salinity of the available water. Such water may effect its influence by way of the gut membranes when ingested or by way of the respiratory membranes when placed into the gill chamber by the thoracic appendages.

In order to study the preference of this species for fresh water and sea water, individual specimens were placed in a situation where both liquids were available. The number of visits to each medium as well as the time spent at each visit were recorded. In the two water containers which were placed at the opposite ends of a large box were constructed platforms which were held at the surface of the water under tension, but which could be depressed by a mass of approximately 75 grams. Each platform was connected to a separate lever-arm marking on a kymograph drum. If an animal visited a container to drink, it found it necessary to depress the platform in order to expose the surface of the water. The movement of the platform was recorded on the drum, indicating the number of times the animal used the given container, the time it entered and the period it remained. The box was placed in a comparatively quiet, dark room where temperature fluctuations were not observed to exceed 5° C. The positions of the water containers were reversed frequently to assure the recording of water preference only. A quantity of coconut was made available to all animals tested.

The results of these studies demonstrate that if both fresh water and sea water are made available to Birgus for an indefinite period, the body fluids of this crab will be moderately hypotonic to normal sea water (table 6). Specimen No. 5, which was given access to both types of water for an unrecorded period before being placed in the above apparatus, entered fresh water more often and stayed longer than it did sea water. This preference was consistent even to the last day's record. This might, therefore, be indicative of the species' behavior in nature. In the case of specimen No. 1, which was placed in the apparatus with a low blood concentration equivalent to 69 per cent sea water, the number of visits to the two containers was almost equal. Although it entered fresh water more often, it spent more time in sea water. Elevation of the body fluid concentration to the equivalent of 88 per cent sea water suggests adjustment through greater consumption of sea water.

On the last day of experimentation with this individual only fresh water was entered, indicating that equilibrium was probably reached and behavior similar to that of specimen No. 5 was proceeding. As would be expected, then, animals of high blood concentrations, such as specimens No. 3 and No. 4, entered fresh water more often and remained in it longer than they did in sea water. Since the osmotic pressure of their body fluids was lowered to moderate levels, it seems that adjustment was attained through a relatively greater consumption of fresh water.

TABLE 6

THE CHOICE BETWEEN FRESH WATER AND NORMAL SEA WATER IN BIRGUS, AND THE CONSEQUENT EFFECT ON THE OSMOTIC PRESSURE OF THE BLOOD

o.p. osmotic pressure; (% S.W.)-equivalent to per cent of normal sea water; F.W.-fresh water; S.W.-normal sea water.

A	specimen number	1	3	4	5
B	days in box	15	8	16	52
C	visits to F.W.	11	14	27	63
D	visits to S.W.	9	8	8	32
E	hours in F.W.	9.8	6.5	14.8	72.0
F	hours in S.W.	17.12	0.78	0.66	8.70
G	mean visits per day to F.W.	0.73	1.75	1.70	1.20
H	mean visits per day to S. W.	0.60	1.00	0.50	0.62
I	mean hours per day in F. W.	0.65	0.82	0.92	1.28
J	mean hours per day in S.W.	1.13	0.01	0.04	0.17
K	o.p. of blood before experiment (% S.W.)	69	118	123	X*
L	o.p. of blood after experiment (% S.W.)	88	89	85	92

*exposed to F.W. and S.W. for several days before experiment.

The evidence, then, suggests that in nature both sea water and fresh water are taken on by Birgus and that they are used in relative amounts which tend to maintain the osmotic pressure of the blood at a moderately consistent level, hypotonic to normal sea water. It should be pointed out, however, that the blood concentrations of Birgus taken in its natural habitat has been reported equivalent to about 64 per cent sea water (Harms, 1932). This may mean that sea water was not readily available to these specimens, for such a concentration is about equal to the blood of the above experimental animals after they had been given access to only fresh water for a prolonged period. It may also mean, of course, that the above experimental conditions induced an abnormal behavior so that the crabs consumed more sea water than they would have normally.

DISCUSSION

Among crabs there is a strong correlation between the ability to regulate osmotically and the ability to live on land. Hyper-osmotic regulation is generally present in those crabs which show hypo-osmotic regulation, and all crabs studied which show some degree of terrestrial behavior give evidence of a degree of both types of regulation.

The question therefore arises as to the function of osmotic regulation in these transitional land forms. The evidence indicates that they have come on land directly from the sea and there is no indication that either they or their ancestors have encountered to any significant degree osmotic stresses in the form of dilute sea water sufficient to exceed the tolerable blood concentrations (e.g., 60 per cent of perfect regulation in 50 per cent sea water)

indicated in the present investigation or in the investigation of Jones (1941). Also data in table 1 indicate that dilute blood is commonly tolerated in nature. It seems doubtful, therefore, that osmotic stresses in the form of dilute sea water would have been sufficient to "force" the evolution of hyper-regulation. What then was the need for hyper-regulation?

Indeed, what is the function of hypo-regulation? It is true that occasionally a tide pool of high salinity may be entered by a crab, for instance, to escape a predator, but it has been the observation of Jones (1941) and the author that tide pools of significantly high salinities are rarely entered by Pachygrapsus, i.e., salinities higher than commonly observed blood values in field-sampled crabs (table 4). It has also been observed in the laboratory that Pachygrapsus shows a strong dislike for concentrated sea water. Thus, it seems that even though the crab may enter a tide pool which is hypertonic to its blood, it would not remain long enough to permit large exchanges to occur between the body fluids and the external medium, being easily able to reach the more tasteful sea close at hand. Since these semi-terrestrial crabs have the ability to regulate in both hypo- and hyper-tonic media and yet probably have not faced these stresses, it seems probable that mechanisms involving these abilities have evolved without osmotic stresses imposing the selective pressure. A striking example of such a phenomenon is the crab Heloecius which inhabits brackish to almost fresh water and yet can regulate strongly in concentrated sea water (Edmonds, 1935).

Jones (1941) was aware of the fact that Pachygrapsus rarely enters water of high salinity. He tried to explain hypo-regulation as a method to prevent high salinities of the evaporating gill fluid from affecting the body fluids, but his explanation fails in light of evidence produced in the present investigation, namely, that the quantity of salt in the small volume of branchial fluid is insufficient to effect a harmful rise in the osmotic pressure of the blood, even though it were totally absorbed.

It might be said that osmotic regulation represents a higher degree of homeostasis which an animal would have to achieve to invade the land, but this statement throws no light on the selective forces involved in the evolution of homeostasis.

Now it was shown above that Pachygrapsus and probably Birgus are capable of absorbing water against a gradient. This mechanism might, in effect, function to conserve water, and this obviously would be of high adaptive value for life on land. It is proposed, therefore, that hypo-osmotic regulation *per se* is not of importance because hypertonic media generally are not entered in nature for extended periods. This ability manifests itself in the laboratory, however, because it involves the same mechanisms of excretion of salts from kidney, gut or gills that are involved with water conservation and for that reason appears generally among crabs exhibiting degrees of the terrestrial habit.

We have seen that hyper-regulation for some unexplained reason always occurs in crabs that can hypo-regulate. Hyper-regulation does not appear to reflect any aerial adaptation parallel to the water conservation consequence

of hypo-regulation. Therefore it apparently has never been immediately adaptive for crabs invading the land directly from the sea, but is only an incidental or necessary accompaniment of the reciprocal mechanism, excretion of salt.

In Birgus, which has probably made the largest and most successful step onto land for the decapod Crustacea, interesting behavioral mechanisms have contributed markedly to this success: A. It can moisten its respiratory membranes by its appendages, thus assuring adequate gaseous exchange. This it can do from small puddles of water without immersing itself. Such a behavior has not been observed nor reported among the semi-terrestrial crabs. Pachygrapsus must have sufficient water to immerse itself in order to live indefinitely. B. It can drink water without having to immerse itself. Thus it can be assured of adequate fluids from small, shallow sources. C. It can control the osmotic pressure of its body fluids by selecting among salt solutions the proper amount of salt. This means that not only is the animal precisely "aware" of its body fluid concentrations, but is also "aware" of a precise means by which it can keep such concentrations constant. The semi-terrestrial crab, Pachygrapsus by the nature of its habitat would not have access to a selection of salt solutions, but a token experiment in the present investigation demonstrated that when this crab is given its choice of land or fresh water it soon dies. Yet given a choice of land or sea water it lives indefinitely.

SUMMARY

1. Terrestrial and semi-terrestrial crabs generally demonstrate the ability to regulate osmotically in dilute and concentrated sea water.

2. Osmotic regulation in terrestrial and semi-terrestrial crabs is not adaptive for aquatic life since osmotic stresses such as dilute and concentrated sea water are rarely experienced by these animals. Besides many such animals drown when completely immersed.

3. The branchial chamber of Pachygrapsus contains a volume of fluid equal to a maximum of about 3 per cent of the body volume. Thus, regulation against branchial fluid made concentrated by evaporation would not be adaptive as proposed by Jones because the total salt therein could not elevate the blood concentration above concentrations normally experienced in nature.

4. The respiratory rate of Pachygrapsus decreases when the crab is desiccated. This explains why the terrestrial behavior of Pachygrapsus is limited to brief periods.

5. Pachygrapsus can absorb water against a gradient when it has been desiccated.

6. The ability to regulate in concentrated sea water (hypo-osmotic regulation) is believed to be a manifestation of a mechanism for the conservation of water, namely, elimination of salts.

7. The mechanism involved in hyper-osmotic regulation seems to be necessary to the mechanism of hypo-osmotic regulation but apparently has no adaptive consequence such as salt elimination.

8. The coconut crab, *Birgus latro*, shows special adaptations to terrestrial life.

a. It can drink water from small puddles.

b. It can keep its respiratory membranes moist by placing water into the gill chamber without becoming immersed.

c. It can control the concentration of its body fluids by selecting water of the appropriate salinity.

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AN ANALYSIS OF THE COLOR PHASES OF THE EASTERN SCREECH OWL, OTUS ASIO, BY THE GENE FREQUENCY METHOD

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A sample of Eastern screech owls, *Otus asio*, may be classified into three basic color types, red, gray, and an intermediate. The evolutionary and ecological differences between the chromatic phases were studied as early as 1893 (Hasbrouck, 1893). From the relative frequencies of the red and gray in a sample of about 3600 owls Hasbrouck concluded that the plumage types were independent of age, sex, or season, and that temperature and humidity are the major influences on the relative distribution of the colors. This study also revealed the occurrence of owls with intermediate color. Another investigator (Martin, 1950) classified the feather colors in a sample of 145 owls in Canada into nine categories grading from red, through intermediate, to gray. He also found no significant relationship between the colors and the sexes.

The present study was undertaken to determine the genetic basis of the color variations in the screech owl using the method of gene frequency analysis. A genetic control of the pigmentation was assumed since plumage coloration in domestic fowl and pigmentation in other animals are well known to be affected primarily by the action of genes.

DESCRIPTION OF THE SAMPLE

The population consists of free-living wild birds. No restrictions were put upon them. The data were obtained by observing the occupants of the many nesting boxes erected at favorable owl nesting sites throughout Ottawa County, Ohio. Both male and female parent phenotypes were recorded, if possible, and the phenotypes of the young were always recorded.

Due to the manner of repeated yearly observations there may be repetitions of specific individuals in the data. All boxes were observed without any selection. Any repeated observation of a color phenotype was made proportional to the frequency of that phenotype in the population. The data are summarized in table 1.

GENETIC HYPOTHESES

The data were analyzed by the gene frequency method to test whether the matings occur at random, and whether the offspring were produced in agreement with each of several genetic hypotheses. The method requires the assumption that the population is in equilibrium with respect to mating type, phenotype, and genotype.

TABLE 1

CLASSIFICATION OF 623 OWLS WITH RESPECT TO COLOR IN SAMPLE FROM OTTAWA COUNTY, OHIO

Class	Red	Inter.	Gray
Pedigreed parents	21	2	101
Pedigreed young	38	7	182
Partial pedigreed parents	22	0	26
Partial pedigreed young	59	5	124
Nestings which failed	14	0	22
	154	14	455

The genetic hypotheses tested were: (1) one pair of genes with dominance, (2) one pair of genes without dominance, (3) multiple alleles, and (4) sex associated inheritance.

One pair of genes with dominance. To test this hypothesis the owls may be classified merely as red or gray, and the difference assumed to be caused by one pair of genes with the gene for red exhibiting dominance over the gene for gray. The intermediate owls may be regarded as misclassified gradations of the red and gray.

Removal of the intermediates from the sample total in table 1 leaves 455 gray and 154 red owls. Since an overwhelming number of matings were gray by gray, in the pedigreed matings (table 2), and since these matings

TABLE 2

MATING TYPES AND PROGENY WITH RESPECT TO COLOR

Mating Type Adults	Number of Matings	Progeny Red	Progeny Inter.	Progeny Gray
1. Red × Red	3 3 failed	10	0	0
2. Red × Gray				
♂ × ♀	5 3 failed	9	0	11
♀ × ♂	9 5 failed	17	2	16
Red × Inter.				
♂ × ♀	0	....	....	....
♀ × ♂	1	2	2	0
Inter × Inter.	0	....	....	....
Inter × Gray				
♂ × ♀	1	0	3	2
♀ × ♂	0	....	....	....
3. Gray × Gray	43 7 failed	0	0	153
Totals, offspring	62 18 failed	38	7	182
Totals, parents	80	35	2	123
Totals		73	9	305

produced only gray offspring, the gray phenotype may be assumed to be due to the recessive gene, and red due to its dominant allele.

Let *R* stand for the dominant gene for red pigment and *r* stand for its recessive allele for gray pigment. Let p be the frequency of *R*, and q the frequency of *r* in the sample, such that $p + q = 1$. Values of q and p may be obtained from the sample so that they are the maximum likelihood estimates of θ and $1-\theta$, the true frequencies of r and R in the population.

The estimates may be based upon the parents only, the offspring only, the pedigreed parents and offspring, or upon the entire sample of 609 owls, omitting the intermediates. The entire sample of 609 appears to be the best choice since there are no marked evidences of heterogeneity within it and since the parents and offspring contain about the same frequencies of red and gray owls. Of 609 owls in the entire sample (omitting intermediates), 455 were gray. The frequency of *r* thus $q = \sqrt{455/609} = 0.864$ and of *R* is $p = 0.136$.

The theoretical mating type frequency, determined by the expansion of the binomial $((1-\theta) + \theta)^4$ using $q = 0.864$ as an estimate of θ, and the observed mating frequences are compared in table 3. The data in this table

TABLE 3

TESTING SPECIFIC MATING TYPES UNDER HYPOTHESIS OF ONE PAIR OF GENES WITH DOMINANCE

Mating Type	Theoretical Frequency	Expected Proportions		Expected Frequency	Observed Frequency
1. Red × Red *RR* × *RR*	$(1-\theta)^4$	0.00034			
2. Red × Red *RR* × *Rr*	$4\theta(1-\theta)^3$	0.00864	0.064	5.0	6
3. Red × Red *Rr* × *Rr*	$4(\theta)^2(1-\theta)^2$	0.05501			
4. Red × Gray *RR* × *rr*	$2(\theta)^2(1-\theta)^2$	0.02751			
			0.378	29.5	22
5. Red × Gray *Rr* × *rr*	$4(\theta)^3(1-\theta)$	0.35045			
6. Gray × Gray *rr* × *rr*	$(\theta)^4$	0.55803	0.558	43.5	50
			1.000	78.0	78
				$\chi^2 = 3.06$	

show that the various kinds of matings occurred with frequencies in agreement with those expected if mating is at random. The computed $\chi^2 = 3.06$. This is smaller than the critical $\chi^2 = 5.99$ at the 5 per cent level of significance for 2 degrees of freedom.

Table 4 contains the test of the observed distribution of young produced by individual mating types compared to their expected distribution. The expected numbers of offspring are found from the relative frequencies of the

TABLE 4

DISTRIBUTION OF YOUNG PRODUCED BY MATING PHENOTYPES UNDER HYPOTHESIS OF ONE PAIR OF GENES WITH DOMINANCE

	Mating	Observed Numbers Offspring		Expected Numbers Offspring		χ^2
		Red	Gray	Red	Gray	
1–3	Red × Red	10	0	7.8	2.2	2.74
4–5	Red × Gray	26	27	28.4	24.6	0.45
6	Gray × Gray	0	153	0.0	153.0	....

matings given in table 3. The data show that in all three cases the observed numbers of offspring occur with frequencies in agreement with those calculated if the difference between red and gray is due to only one pair of genes with red dominant to gray. The computed χ^2 for mating types 1–3 equals 2.74, a smaller value than the critical $\chi^2 = 3.84$ at the 5 per cent level of significance for 1 degree of freedom. The computed χ^2 for mating types 4 and 5 equals 0.45, and for mating type 6 equals 0.0. Both of these are smaller than the critical χ^2 value 3.84 at the 5 per cent level of significance for 1 degree of freedom.

One pair of genes without dominance. Test of the hypotheses that the feather colors are inherited in a genetic system lacking dominance reveals specific inconsistencies. First, consider a system in which *RR* determines an intermediate phenotype, *Rr* determining a red phenotype and *rr* determining the gray phenotype. A crucial test mating would involve an intermediate and a gray owl. If the hypothesis were correct this mating should produce nothing but red offspring. The one observed mating in this category (table 2) produced both intermediate and gray, but none of the required phenotype. The frequencies among the progeny produced by the red × red matings (table 2), tested against the theoretical expectations of ¼ intermediate, ¾ red, and ¼ gray, yield a χ^2 of 10.0. This is greater than the critical $\chi^2 = 5.99$ at the 5 per cent level of significance for 2 degrees of freedom. This is evidence against the hypothesis.

Second, consider a system in which *RR* determines the red phenotype, *Rr* the intermediate, and *rr* the gray. A mating of red and gray should produce nothing but intermediate owls. The evidence in table 2 is contrary to this also since only 2 intermediate owls were produced of the 55 expected from matings of this type. These are the only possibilities since gray must be due to a homozygote in order always to produce gray when mated to similarly colored individuals.

Multiple Alleles. Another possible mode of inheritance is a multiple allelic system. Assume the red, intermediate, and gray phenotypes are inherited in this manner with a graded order of dominance of *Y* (red) dominant to *y′* (intermediate) dominant to *y* (gray). Table 5 illustrates the observed and expected sample frequencies and the theoretical population frequencies used in deriving the values for the statistics $r = 0.1324$, $s = 0.0120$, and $t = 0.8546$, as estimates of the true frequencies of genes *Y*, *y′*, and *y*.

TABLE 5

SAMPLE FREQUENCIES AND ESTIMATES OF POPULATION GENE FREQUENCIES UNDER HYPOTHESIS OF A SYSTEM OF MULTIPLE ALLELES

Genotype	Theoretical Frequency	Observed Frequency	Expected Frequency
YY	ρ^2		0.018
Yy'	$2\rho\sigma$	0.247	0.003
Yy	$2\rho\tau$		0.266
$y'y'$	σ^2		0.0002
$y'y'$	$2\sigma\tau$	0.023	0.022
yy	τ^2	0.730	0.730

$t = \sqrt{455/623} = 0.855$, frequency of y

$s = \sqrt{0.753} - 0.855 = 0.013$, frequency of y'

$r = 1 - (t + s) = 0.132$, frequency of Y

The theoretical mating type frequency determined by the expansion of the trinomial $(\rho + \sigma + \tau)^4$ and the observed mating type frequencies are compared in table 6. The data in this table show that the various kinds of matings occurred with frequencies in agreement with those expected if mating is at random. The computed $\chi^2 = 4.26$. This is smaller than the critical $\chi^2 = 11.07$ at the 5 per cent level of significance for 5 degrees of freedom, therefore the matings are at random.

TABLE 6

TESTING SPECIFIC MATING TYPES UNDER HYPOTHESIS OF A SYSTEM OF MULTIPLE ALLELES

Mating Type by Phenotypes	Theoretical Frequency	Expected Proportions	Expected Frequency	Observed Frequency
1. Red × Red	$\rho^4 + 4\rho^3\sigma + 4\rho^3\tau + 4\rho^2\sigma^2 + 8\rho^2\sigma\tau + 4\rho^2\tau^2$	0.061	4.9	6
2. Red × Gray	$2\rho^2\tau^2 + 4\rho\sigma\tau^2 + 4\rho\tau^3$	0.361	28.9	22
3. Red × Inter.	$2\rho^2\sigma^2 + 4\rho\sigma^3 + 12\rho\sigma^2\tau + 4\rho^2\sigma\tau + 8\rho\sigma\tau^2$	0.011	0.9	1
4. Inter. × Inter.	$\sigma^4 + 4\sigma^3\tau + 4\sigma^2\tau^2$	0.001	0.1	0
5. Inter. × Gray	$2\sigma^2\tau^2 + 4\sigma\tau^3$	0.033	2.6	1
6. Gray × Gray	τ^4	0.533	42.6	50
		1.000	80.0	80

$\chi^2 = 4.26$

Table 7 shows the observed distribution of young produced by the specific phenotypic mating types as compared to their expected distribution.

The data show agreement in all but one case between the frequencies of the observed numbers of offspring and those calculated if the differences between red, intermediate, and gray were due to a multiple allelic genetic system. This system involves a graded order of dominance of red over intermediate over gray. The computed χ^2 for matings of type 1 is 2.76, a

TABLE 7

DISTRIBUTION OF YOUNG PRODUCED BY MATING PHENOTYPES UNDER HYPOTHESIS OF A SYSTEM OF MULTIPLE ALLELES

Mating	Observed Numbers Offspring			Expected Numbers Offspring			χ^2
	Red	Inter.	Gray	Red	Inter.	Gray	
1. Red × Red	10	0	0	7.8	0.1	2.1	2.76
2. Red × Gray	26	2	27	29.4	0.4	25.2	7.38
3. Red × Inter.	2	2	0	2.1	1.0	0.9	1.97
4. Inter. × Inter.	....	....	....	....	....	....	....
5. Inter. × Gray	....	3	2	....	2.5	2.5	0.19
6. Gray × Gray	....	....	153	....	....	153.0	....

smaller value than the critical $\chi^2 = 5.99$ at the 5 per cent level of significance for 2 degrees of freedom. Similarly the calculated $\chi^2 = 1.97$ for matings of type 3 is smaller than $\chi^2 = 5.99$ for 2 degrees of freedom at 5 per cent level, and $\chi^2 = 0.19$ for matings of type 5 is smaller than $\chi^2 = 3.84$ for 1 degree of freedom at the 5 per cent level of significance. The one significant computed $\chi^2 = 7.38$ for matings of type 2 is greater than the critical $\chi^2 = 5.99$ at the 5 per cent level for 2 degrees of freedom. On the surface one would reject the hypothesis, but deeper scrutiny shows that 68/74 of the value of χ^2 is contributed by the intermediate class. In this class we observed 2 individuals whereas but 0.382 was expected. The significant χ^2 could be due to the small sample size or to the uncertainty of classification. If we group the intermediate individuals with the red category we obtain a computed $\chi^2 = 0.58$. This is much smaller than the critical $\chi^2 = 5.99$ at the 5 per cent level of significance for 2 degrees of freedom. Similarly when regrouping the intermediates with the grays the computed $\chi^2 = 1.37$ which is also much smaller.

Sex associated inheritance. If the difference between red, intermediate, and gray is due to one of the sex associated modes of inheritance we would not expect the owl color to occur independently of the sex. Sex and color are apparently independent attributes (table 8). The calculated χ^2 value of

TABLE 8

CONTINGENCY TABLE TESTING THE INDEPENDENCE OF OWL COLOR AND SEX

Color	Male	Female	Totals
Red	14	21	35
Inter.	2	0	2
Gray	64	59	123
Totals	80	80	160

$\chi^2 = 3.60$

3.60 is less than the critical value for χ^2 of 5.99 at the 5 per cent level of significance for 2 degrees of freedom. Since color and sex are independent of each other the genes controlling the color phases have no association with the sex associated modes of inheritance.

DISCUSSION

This analysis includes all modes of inheritance for one gene locus. There appears to be no need to consider more than one gene locus. Lack of dominance may be dismissed as an acceptable mode of inheritance since neither of the ways in which this mode could be applied could account for the distribution of colors of the young produced. None of the sex-associated modes are acceptable since the two attributes, color and sex, are independent of each other. Martin (1950) arrived at the same conclusion in his study on *Otus asio* in Ontario, Canada. This leaves but two of the modes of inheritance considered here as possible explanations of the observed data. In testing the assumption of simple dominance, it was noted that red by red produced only red. This can be attributed to the small sample size analyzed. Agreement with the hypothetical expectations of finding some red by red matings which produce all red, some mixed, and a few producing all gray was noted in Hasbrouck's (1893) data. Under the hypothesis of multiple alleles, the observed value of the intermediate class from type 2 matings (table 7) deviates from the hypothetical value by a significant amount. The small sample size may well account for this deviation. When the numbers of this class were grouped with each of the other classes, the χ^2 arriving from each of these distributions was smaller than the critical value. The results of one mating cannot be explained by this hypothesis. This mating involves a red by a gray producing 2 red, 1 gray, and 2 intermediate. This is an impossible outcome under this hypothesis, barring mutation, and also adds weight to its rejection. In this type of a mating only two phenotypes can possibly be produced. This may be reconciled as a misclassification. However if we accept this reasoning then the entire intermediate class could be considered as such.

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SUMMARY

A satisfactory agreement was obtained between the breeding data and two of the hypotheses of gene action:

1. If the intermediate phenotype is assumed to be due to genetic modifiers, the difference between red and gray is due to one pair of alleles, the gene for red exhibiting dominance over its allele for gray.

2. If all three colors are considered, the colors are inherited on the basis of a series of three alleles with a graded order of dominance of red → intermediate → gray.

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A PRELIMINARY COMPARISON OF THE FERTILITIES OF WILD HOUSE MICE WITH AND WITHOUT A MUTANT AT LOCUS T.

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Many populations of wild house mice in the United States contain animals heterozygous for a mutant allele T ($+/t^w$), at locus T as well as animals without such an allele ($+/+$). Of seven such alleles, found in wild populations and tested in the laboratory, five are lethal and two are viable in homozygotes (t^w/t^w) (Dunn and Morgan 1953; Dunn 1955). In all of these cases, females heterozygous for such an allele show normal segregation, that is, 50 per cent of t^w gametes are produced; but male heterozygotes always produce a great excess of t^w offspring, up to 95 per cent (Dunn and Morgan 1953). It has been estimated that a mutant allele with such a segregation-ratio advantage in the male, should spread through a wild population even when lethal, reaching a limiting value of .5 $+/t^w$ (Prout 1953). The small samples of mice which have been tested from several wild populations have in general yielded fewer $+/t^w$ heterozygotes than would be expected on the above assumption while in some populations no t-alleles have been found. It has thus become necessary to seek for effects of t^w in heterozygotes which might serve to counterbalance the advantage which t^w alleles enjoy in segregation.

As a first step, the number of litters and the number of offspring per litter over a standard test period have been recorded for $+/+$ and $+/t^w$ males and females from a population of wild mice bred for several generations in the laboratory. The experiment was arranged as follows: Females from this population, known from progeny tests to be $+/t^w$, were mated with males known to be $+/+$. The resulting offspring were mated at the age of 4–8 weeks with mice of genotype $T/+$ (Brachy i.e. short-tailled) from a standard laboratory stock (H Br) derived from a cross between two different Brachy inbred lines. The females, $+/+$ and $+/t^w$, were mated, eight to a large pen with an exercise wheel, with a Brachy male previously tested and known to be fertile; each male ($+/+$ or $+/t^w$) was placed in a pen with four Brachy females which had previously been tested for fertility by having had a litter of at least five offspring. Females which became pregnant were removed from these pens a few days before the birth of the litter (in an individual isolation pen). In the pens in which males were being tested, females removed were immediately replaced by fresh females so that four females were always available to each wild male. These tests of wild females and of fertile wild males were continued for eight months; in the case of males which failed to breed, fresh fertile females replaced the original females

*Work carried out at Nevis Biological Station, Irvington-on-Hudson, N.Y. The cooperation of Mr. Robert E. Stephenson, superintendent of Nevis, and the assistance of Miss Mary Ann Dann is gratefully acknowledged.

from time to time until each such male had accumulated 12 test-mating units. (A mating unit is exposure to one fertile female for one month.) Failure to induce pregnancy during 12 mating units resulted in classification of the male as sterile.

Litters born from these matings were scored for the following phenotypes at birth: tailless T/t^w, Brachy $T/+$, and normal tail. Wild mice which produced tailless offspring were classified as $+/t^w$; those which produced only Brachy and normal (test requirement 12 offspring) were classified as +/+.

The results of these tests are shown in table 1.

DISCUSSION

Table 1 contains the records of 25 males which have completed at least 12 mating units, equivalent to a pair mating for one year. Of these, 19 were classified as $+/t^w$, 4 as +/+, and 12 as sterile. Since the genotypes expected (from cross of $+/t^w$♀ × +/+♂) were .5 $+/t^w$ and .5 +/+, it is evident that the deficiency of +/+ males found is probably to be accounted for by the inclusion of many of this genotype in the sterile class. If all sterile males are +/+, the ratio becomes 19 $+/t^w$ to 16 +/+, close to that expected.

Table 1 (below) contains comparable data for the females. Here the differences in the frequencies of $+/t^w$ and +/+ are probably not significant.

TABLE 1

RESULTS OF TESTING WILD MALES (FROM MATING $+/t^w$ FEMALE BY +/+ MALE) BY BRACHY $T/+$ FEMALES

Geno type	No. of litters	Mating units	Offspring				Total	Total* mating units
			Normal +/+ $+/t^w$	Brachy $T/+$	Tailless T/t^w	Unclassified		
21 $+/t^w$ ♂♂	254	487	914	123	786	21	1844	3.78
4 +/+ ♂♂	59	131	229	204	....	7	440	3.35
12 ♂♂ (sterile)	....	144	....	....	....	....	....	....
10 $+/t^w$ ♀♀	49	80	158	86	74	10	328	4.10
8 +/+ ♀♀	39	64	144	117	0	4	265	4.14
11 +/+ ♀♀ (sterile)	....	88	....	....	....	....	....	....

*This gives the net relative fertility, i.e. number of offspring per mating unit of one month.

The ratio of tailless to Brachy offspring from $+/t^w \times T/+$ expresses the segregation ratio of t^w: + gametes in the $+/t^w$ parent. From $+/t^w$ females this ratio is .46, not significantly different from the normal .5; from $+/t^w$ males the ratio is .86, indicating a great excess of sperm transmitting t^w, as is usual in wild heterozygotes.

Our experience leads us to believe that the tests of the males more accurately reflect genotypic differences in fertility than those of the females.

Wild females usually do not breed unless supplied with exercise wheels (Schneider 1946). Although such exercise wheels were provided for all breeding pens, we have at present no way of knowing the extent to which all females in the pen used the wheel. The occurrence of oestrous in the female is sensitive to many environmental variables (light and dark, temperature, disturbance and others) which were not rigidly controlled, although all pens were treated alike. Consequently from this preliminary experiment we derive only the hypothesis that more heterozygote males ($+/t^w$) are fertile, under the conditions of the laboratory tests, than homozygous normals +/+.

Since the differences are in the direction opposite to those which would supply the selective factor counterbalancing the segregation-ratio advantage of t^w, it is evident that the hypothesis must be tested in more extensive experiments which can be subjected to statistical analysis, and in which other elements contributing to the relative adaptive values of the two genotypes can be measured. Such experiments are under way.

SUMMARY

The relative fertilities of two genotypes of mice from a wild population have been compared by breeding them in the laboratory to a standard inbred stock. Males heterozygous for a lethal ($+/t^w$) appear in a preliminary test to be superior to those without the mutant allele (+/+). The hypothesis of heterozygote superiority is being tested further.

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NATURAL SELECTION AND THE OUTCOME OF COMPETITION*

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Several studies of competition between two species of flour beetles (*Tribolium confusum* and *Tribolium castaneum*) have led to two primary qualitative conclusions: (1) single-species populations persist indefinitely, the numerical level of their persistence being correlated with the ecological conditions in which they are husbanded and (2) mixed-species populations invariably display, in consequence of coassociation, survival of one form and elimination of the other. Survival and elimination thus emerge as the "criteria" of competition and it is reasoned that the underlying causation originates from interaction between the species. These phenomena are discussed, both in their biological and statistical aspects, by Park (1948), Kennington (1953), Park (1954, 1955) and Neyman, Park, and Scott (1956). On *a priori* grounds the causation responsible for such eliminations must be ecological and/or genetic. The present paper is devoted to a simple but time-consuming experiment which suggests that stringent natural selection need not be invoked in explaining the outcomes attributed to the competition process.

Many of the competition studies referred to above have been conducted in similar fashion. A small vial receives 8 grams of a medium consisting of 95 per cent whole-wheat flour and 5 per cent dry yeast and into this are simultaneously introduced 2 male *T. confusum*, 2 female *T. confusum*, 2 male *T. castaneum*, and 2 female *T. castaneum*. The vial is then placed in a dark incubator which maintains the approximate climate of 29°C., and 70 per cent relative humidity. The culture, along with its battery of replicates, is undisturbed for 30 days at which time the flour is sifted, the living stages are counted and recorded, and the entire population is returned to 8 grams of fresh medium, in the same incubator, for 30 additional days. Such procedures are sustained until well after that time when one species has disappeared—an interval which for some experimental designs may exceed three or four years.

As shortly to be documented, this treatment results in the consequence that *T. castaneum* usually survives (that is, significantly greater than one-half the time) while *T. confusum* is eliminated. However, with lower incidence the obverse holds true, namely: *T. confusum* is the survivor. Data for the present study were got by using *T. confusum* from the latter, or infrequent, case histories. The possibility exists that gene-frequency changes occurred in each of these *T. confusum* populations through natural selection induced by competition and such changes, if real, could enhance the probability of this species' survival.

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To explore the matter we erect a hypothesis and subject it to test. The hypothesis is as follows: Ability to compete can be selected, and, when *T. confusum* is the winner, this selection has been so great that it has caused the observed reversal of the usual outcome of competition. If selection has indeed been this strong then the prediction seems permissible that later descendants of these populations should win more frequently when in competition, and/or persist longer if eliminated, than do *T. confusum* from the stock culture. This prediction was tested experimentally by taking random samples of *T. confusum* from those four populations in which earlier it had been the victor and pitting them against an equal number of *T. castaneum* from original stock.

Specifically, this is what was done. In the experiments reported by Park (1954) 28 competition cultures were established in a 29°-70 per cent environment. Of these, there were 24 replicates in which *T. castaneum* survived and four in which *T. confusum* survived. In these four latter replicates, extinction of *T. castaneum* had occurred at the respective ages of 300, 330, 480 and 570 days, the mean being 420 days. Since the minimum generation time is about 30 days at 29°C., the maximum number of generations is 14. At about the nine-hundredth day, after an additional 11 to 20 generations had elapsed, a group of pupae were chosen at random from each culture. These were sexed, allowed to emerge as young adults, and two pairs (again at random) were placed in a standard vial. At the same time two other pairs of young *T. castaneum*, but derived as pupae from *stock* culture, were also put into the vial. Ten such populations were started in such a way that the first three replicates received their *T. confusum* from one original population (number-7); the next two, from number-17; the next two, from number-21, and the final three, from number-24. A census of each of the ten was taken in the usual way each 30 days until extinction of one species occurred.

Table 1 pertains to the first prediction from hypothesis, namely: the

TABLE 1

RECORD OF SPECIES-ELIMINATIONS IN COMPETITION POPULATIONS BASED ON FOUR INDEPENDENT INVESTIGATIONS

Items	Sources				
	Park 1948	Kennington 1953	Park 1954	Present study	Combined
Number of populations	18	20	28	10	76
Number of *T. confusum* eliminations	12	19	24	9	64
Number of *T. castaneum* eliminations	6	1	4	1	12
Percentage of *T. confusum* eliminations	67	95	86	90	84
Percentage of *T. castaneum* eliminations	33	5	14	10	16

percentage of eliminations of *T. castaneum* in the present study should exceed that of previous experience. From the table the following points merit attention:

(1) *T. castaneum* is the consistent winner.

(2) The frequency of wins of *T. castaneum* over *T. confusum* departs significantly from an assumption of even-chance distribution of winners to losers in every study except Park (1948). But the latter is in the same direction (67 per cent to 33 per cent in favor of *T. castaneum;* P = 0.13) and the inference therefore seems plausible that this difference is also real.

(3) When all data are combined thereby yielding the impressive replication (for studies of this sort) of 76 complete population histories, *T. confusum* survives in only 16 per cent of the cases and this departure from an expectation of ½:½ is highly significant (P<0.0000).

(4) There is no evidence that the populations comprising the present study yield an end-result which differs either qualitatively or quantitatively from the others. If the percentage eliminations of *T. castaneum* are ranked from least to most they run 5, 10, 14, and 33 per cent. The present study is identified by the 10 per cent value—a value higher than one but lower than the other two figures and not appreciably different from the mean of the total array.

From the last point we conclude that the *a priori* prediction is not fulfilled; or, that the hypothesis fails to find empirical confirmation.

The second aspect of the study deals with temporal relations rather than with species-frequencies and is summarized in table 2. Here we expect,

TABLE 2

POPULATION-AGES AT WHICH SPECIES-ELIMINATIONS OCCUR (DAYS)

Source	Criteria	*T.confusum* eliminated	*T.castaneum* eliminated
Park, 1948	First elimination	510	210
	Median	660	315
	Last elimination	840	450
Kennington, 1953	First elimination	300	480
	Median	390	480
	Last elimination	660	480
Park, 1954	First elimination	390	300
	Median	645	405
	Last elimination	1410	570
Present study	First elimination	210	390
	Median	570	390
	Last elimination	930	390
Combined	First elimination	210	210
	Median (averaged)	566	398
	Last elimination	1410	570

according to hypothesis, that the "selected" *T. confusum* progeny used in the present study, though eliminated with the usual frequency by *T. castaneum*, will nonetheless persist longer when in competition. Again, we compare the four studies but choose three criteria for each that describe the population-age at which one species disappears owing to the sustained action of competition. The criteria are time (in days) of the *first* elimination, of the *last* elimination, and the *median* of each array of comparable cases.

The table permits a number of inferences to be drawn and among these should be mentioned the following:

(1) The range and value of ages at first elimination are approximately the same whether one, or the other, species dies out.

(2) The values of median ages, and ages at last extinction, are greater for *T. confusum* than for *T. castaneum* even though the former is the more frequent loser. This is true for all four studies and the point was commented upon in more detail elsewhere (Park, 1954).

(3) There is no suggestion that the ages of elimination of *T. confusum* in the present study are postponed beyond those in the previous studies. In respect of the first elimination the present study has rank-1 (of four); for the median, rank-2; for the last elimination, rank-3.

(4) The median age for the present study is closest to the averaged median for the combined array (570 cf. 566 days). The median for that one instance in which *T. castaneum* became extinct is also essentially identical to the median of *its* array.

Again we assert, but with certain reservations shortly to be advanced, that the hypothesis is not substantiated. This is to say, any natural selection which occurred during competition was probably not of sufficient intensity to cause a reversal of that outcome typically seen when two species share the same resource.

It is important to recognize, however, that we are concerned in all these studies with populations that are relatively small. Wright (1931) has shown convincingly that any systematic natural selection is necessarily modified by random errors—errors that are chiefly an inverse function of the size of the effective breeding population. It is not our intent, nor indeed are we competent, to discuss this phase of the problem to any degree. But it does seem appropriate to present sufficient information about population size to enable the reader, if so inclined, to form some impression about the magnitude of genetic-drift which reasonably might be expected in such populations as these. To this end we include table 3 in which appears, both during and

TABLE 3

AVERAGE ADULT DENSITIES OF *TRIBOLIUM CONFUSUM* IN THE FOUR REPLICATES IN WHICH *TRIBOLIUM CASTANEUM* WAS ELIMINATED. (FROM PARK, 1954)

Replicate number	Competition Interval (day-60 on)			Post-competition Interval		
	Arithmetic Mean	Harmonic Mean	N	Arithmetic Mean	Harmonic Mean	N
7	81	70	14	151	139	14
17	93	89	9	150	136	19
21	105	102	8	106	103	20
24	66	43	17	123	114	11
Averages	86	76		132	123	

subsequent to competition, the averaged population numbers of those four cultures characterized by survival of *T. confusum* (from Park, 1954). The averages can be taken as *maximum* estimates since effective population sizes doubtless were smaller.

With reference to the present study an argument something like this can be advanced. If random errors are large with respect to selection then it does not seem likely that selection, acting alone over a period of a few generations, would be responsible for genetic effects of sufficient degree to reverse the expected outcome of competition. Even so, it is possible that large *random* genetic changes, taking place in *either* species during the competition-interval, might be related in some measure to that success infrequently enjoyed by *T. confusum*. It is not unlikely that deleterious alleles might have been fixed in the losing *T. castaneum* populations very early in the experiment, although the present study affords no evidence on this point. What the evidence *does* suggest is that competitively superior genotypes, whether induced by selection or drift, did not appear in the *T. confusum* populations in those four replicates in which the latter bested its rival.

A major weakness of this conclusion must be recognized however and lies in the fact that the present study was begun, not immediately after elimination of *T. castaneum*, but rather postponed for 11 to 20 generations. The experimental history encompasses on the average some 14 generations exposed to the risk of selection and random processes. This is followed by as many generations during which time only random errors, or possibly even reverse selection, could have taken place with respect to alleles concerned with interspecies competition. Now, if random errors are large with respect to selective pressures, it is possible that any genetic effects produced in the *T. confusum* generations by selection and/or genetic drift during the competition-interval might have been dissipated by such drift prior to the time when the present cultures were started. This would not be expected to occur in *every* replicate, however, since drift, by itself, would be as likely to increase such genetic effects as to reduce them. Because of these considerations it has not been conclusively proved, therefore, that the four winning *T. confusum* populations were not competitively superior to the stock culture at the end of the competition-interval, although it has been shown that this was not the case some 16 generations later.

SUMMARY

The present study suggests that when two small populations compete for the same resource and one of them is eliminated after a few generations, natural selection resulting from competition between the two is not likely to have had appreciable effects in determining the outcome. This should not be interpreted as evidence that *random* genetic changes taking place during this period cannot affect the outcome of competition, nor obviously, that natural selection resulting from interspecies competition may not be important in evolution.

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THE MUTAGENIC ACTION OF PYRONIN-B

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Clark (1953) found that pyronin-B, when added to the food of *Drosophila melanogaster* larvae, produces mutations. Quantitatively, the effect was weak, only 1.2 per cent sex-linked lethals being found in 2000 tested chromosomes. In similar tests with formaldehyde, up to 15 per cent sex-linked lethals may be obtained. Among possible reasons for this difference in mutagenic efficiency the following had to be considered; Sensitivity to the mutagenic action of formaldehyde food is restricted to one particular stage in the larval testis (Auerbach and Moser 1953). As a result, males which have spent their larval life on formaldehyde food yield high mutation rates only during the first few days of their reproductive period, when sperm treated during the sensitive stage is being utilized. Subsequently, mutation rate falls steeply, the rate of decline depending on opportunity and stimulus for sexual activity. In general, control level is reached as early as 6 days after the first mating. Concomitantly with the decrease in mutation rate, fertility, which is low during the first days, becomes restored to normal; it is therefore usually not possible to test as many chromosomes from the first brood, in which mutations are frequent, as from later broods, in which mutations are rare or absent. Hence, conclusions on the mutagenic efficiency of formaldehyde food which are based on pooled results from the first 10 days of mating would lead to a very serious underestimate of the effect on responsive cells. If the mutagenic action of pyronin feeding were similarly limited to a particular stage, Clark's technique of collecting progeny for testing over 10 days might well have obscured a much more drastic action on sensitive cells. This possibility can be tested by rearing Drosophila larvae on pyronin-food and determining mutation rates in successive broods from treated animals.

There is a further reason why such tests are of interest. The observation that formaldehyde food produces mutations almost exclusively during the growth stage of the primary spermatocyte (auxocyte) has been taken to suggest that the mechanism of mutagenesis in this case consists of interference with some synthetic process (Auerbach and Moser 1953). On the other hand, it seemed possible that for some inherent reason this particular cell type responds much more readily than other larval germ cells to whatever mutagenic influences impinge on it. Khishin's investigations (in the press) have ruled out this possibility for X-rays as the mutagenic agent. It still might exist for mutagenic chemicals which have to reach the germ cells *via* the digestive tract. Pyronin offered an opportunity for testing this.

MATERIAL AND METHODS

The treated males came from a wild-type Oregon stock (Or-K), which over many years has been used for mutation experiments in this laboratory. Its spontaneous mutation rate to sex-linked lethals in males varies from 0.1 to 0.3 per cent and hardly ever goes as high as 0.5 per cent. Frequencies approaching or exceeding 1 per cent for sex-linked lethals and 2 per cent for lethals on the second chromosome can confidently be attributed to an effect of the treatment. The spontaneous mutation rate for ♀♀ of this stock is less well known; in those experiments in which it was taken it did not exceed 0.2 per cent.

The food to which pyronin was added contained dextrose, sugar and dead yeast in two experiments, and molasses, maizemeal and yeast in the third. As, contrary to the experience with formaldehyde, this difference in the composition of the food did not seem to influence the result, it can be disregarded. Contrary to Clark's experience, pyronin-B proved very toxic to the larvae, so that 0.8 per cent was the highest concentration tolerated. Even with this concentration, survival was low and development considerably delayed.

Surviving ♂♂ were mated individually to three virgin females, and remated to a second batch of females four days later. After 7 or 8 days the males were discarded. In experiments with formaldehyde food this breeding procedure would have resulted in a steep decline of mutation rate from the first to the second brood. In two experiments, sex-linked lethals were scored by the Muller-5 method; in one, autosomal lethals were scored by the *Cy/L* method. The tests for sex-linked lethals were carried out on both treated males and females; autosomal lethals were scored only in the progeny of treated males. Autosomal lethals from the same male were cross-tested for identity; this allows the detection of bunches of mutations and, since bunches of more than two lethals must have arisen in premeiotic cells, this gives some indication of the time of origin of the mutations.

RESULTS

Of the two tests for sex-linked lethals, one gave an entirely negative result for both sexes. The results of the other are presented in table 1. There was a marked heterogeneity in the distribution of the lethals over the

TABLE 1

THE FREQUENCY OF SEX-LINKED LETHALS IN THE PROGENY OF 21 MALES AND 19 FEMALES WHICH HAD BEEN REARED ON FOOD CONTAINING 0.8 PER CENT OF PYRONIN-B

Days after first mating	Males			Females		
	n	lethals	%	n	lethals	%
1–4	400	5	1.3	349	3	0.9
5–7	407	5	1.3	427	2	0.5

n = number of tested chromosomes

treated individuals. In the male series, in which about 20 chromosomes were tested per male per brood, 18 males had no lethal, one male had one, one male had 2, and one male had 7. In the female series, with a similar number of tested chromosomes, 2 out of 5 lethals occurred in the same female, one in each of her two broods.

The result of the test for autosomal lethals is shown in table 2. Heterogeneity between males was as pronounced as in the experiment on sex-

TABLE 2

THE FREQUENCY OF LETHALS ON THE SECOND CHROMOSOME OF 49 MALES WHICH HAD BEEN REARED ON FOOD CONTAINING 0.8 PER CENT OF PYRONIN-B

Days after first mating	n	Lethals	Per cent lethals	Semi-lethals	Visibles	Per cent of all mutations
1–4	462	9	1.9	2	2	2.8
5–8	432	8	1.8	2	1	2.5

n = number of tested chromosomes

linked lethals. Some, but not all of it, could be attributed to the occurrence of bunches of identical mutations. 39 males with altogether 726 tested chromosomes had no mutation. Six males with altogether 89 tested chromosomes had one lethal each. One male had one full lethal and a bunch of two identical semi-lethals in 19 tested chromosomes. One male had a bunch of 4 identical lethals in 20 tested chromosomes; the chance that the lethal had not arisen in the experiment, but had been present previously in heterozygous condition, is only about one per cent. One male with 20 tested chromosomes had a bunch of two identical lethals, a further full lethal, and a semi-lethal with visible effect on the wings.

Finally, all three visible mutations were to *vg* and occurred among 20 tested chromosomes of a male, which in addition had one semi-lethal and 3 full lethals, all different from each other.

DISCUSSION

These results confirm Clark's claim that pyronin-B, when added to the food of Drosophila, produces mutations in the larval testis. It is true that the overall effect is small and that in one experiment the result was even quite negative. However, the repeated occurrence of several different mutations in the same male makes it certain that there has been an actual induction of mutations and not just a slight speeding-up of the spontaneous mutation process through some more generalized physiological effect. The data on females are less convincing; but the overall mutation rate in both broods is high for females, and this together with the occurrence of 2 lethals in the same female points to a mutagenic action also on oogonia.

The very marked heterogeneity between males indicates that some very special conditions have to be fulfilled before the treatment can produce mutations; once they are fulfilled, the effect may be drastic, as shown by some of the treated males which had several different mutations in small

progenies. It might be possible to increase considerably the mutagenic overall effect of pyronin-feeding, if the conditions favoring its action were known. At present they are quite unknown; they may be connected with the micro-environment of the feeding larva or with the physiological state of the larva itself. They do not, as with formaldehyde-feeding, include the existence of a restricted zone of sensitivity in the larval testis; this follows from the similarity of mutation rates in successive broods.

There are still further differences between the action of formaldehyde and that of pyronin in the food of Drosophila. Whilst formaldehyde acts only or mainly at such a late stage of germ cell development that the multiplication of an induced mutation into a bunch of more than two is precluded, and that even bunches of two are exceedingly rare, pyronin can also attack the chromosomes of young spermatogonia, as shown by the frequent production of bunches of up to three or four identical mutations in small progenies. Whilst formaldehyde is quite ineffective on female germ cells, pyronin seems to produce mutations even in oogonia.

Thus, although both formaldehyde and pyronin have to reach the germ cells *via* the food, they elicit quite different patterns of response in the larval gonads. Penetration can hardly be responsible for this. It is true that for reasons of chemical specificity the small formaldehyde molecule—or some compound formed between it and a constituent of the food—might be excluded from cells into which the much larger pyronin molecule can penetrate; but genetical tests show that formaldehyde reaches the chromosomes in the spermatocyte, where they are protected by a very much thicker layer of protoplasm than in the young spermatogonium, and tracer studies by Kaplan (unpub., quoted by Auerbach and Moser 1953) have shown that at least the carbon atom of formaldehyde enters equally well into mutation-resistant as into mutation-sensitive cells. The hypothesis that the mutagenic action of formaldehyde is somehow bound up with the synthetic processes which take place during preparation for meiosis receives therefore some support from the finding that the auxocyte stage shows no preferential sensitivity to every mutagen administered in the food.

SUMMARY

Drosophila melanogaster males which as larvae had been reared on food containing 0.8 per cent of pyronin-B were mated individually to two successive batches of females at an interval of four days, and recessive sex-linked and autosomal lethals were scored in their progenies. There was a definite mutagenic effect of the treatment, weak in the overall results, but very high in some of the treated males. This marked heterogeneity shows that some very special conditions of micro-environment or physiological state of the larva are required for the treatment to be effective. In contrast to formaldehyde-food, pyronin-food produces similar mutation rates in successive broods and tends to induce bunches of identical mutations. This shows that its action is not, like that of formaldehyde-food, restricted to growing spermatocytes, but extends backwards into young spermatogonial

stages. It also seems to produce mutations in oogonial chromosomes, which are refractory to formaldehyde. The implications of these differences between pyronin and formaldehyde are discussed.

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LETTERS TO THE EDITORS

Correspondents alone are responsible for statements and opinions expressed. Letters are dated when received in the editorial office.

INBREEDING, GENES AND PHENOTYPES

Glass (1954) has recently commented on one of our earlier papers (Sanghvi & Khanolkar, 1949) on data relating to seven genetical characters in six endogamous groups in Bombay. He says, "In an analysis of this kind, the finding of significantly different phenotypic frequencies cannot be regarded as sufficient to demonstrate the existence of differences in the frequencies of alleles. The possible existence of different degrees of inbreeding in the populations studied must also be reckoned with, for obviously a higher amount of inbreeding will increase the frequency of the recessive phenotypes and conversely decrease those of the dominant phenotypes without itself introducing any change in allele frequencies. If the amount of inbreeding is not negligible, the customary formulas for deriving allele frequencies from phenotypic frequencies yield false results." While in agreement with this general statement, the following data (Table 1) will show how negligible is the actual effect of inbreeding on recessive phenotypes for common characters like the ones under consideration. The values of co-efficient of inbreeding selected for the purpose are $\alpha = .001$ (near about the observed values for some European populations, Orel (1932), Haldane & Moshinsky (1939)) and $\alpha = .01$ (near about the observed values for some of the endogamous groups in Bombay, Sanghvi, (1954)). The values of gene frequency have been taken to be from 0.01 to 0.7 which covers the range encountered in the above mentioned data.

TABLE 1

	% of Recessive Homozygotes		
Gene frequency	Random mating	$\alpha = .001$	$\alpha = .01$
0.70	49.00	49.021	49.21
0.50	25.00	25.025	25.25
0.30	9.00	9.021	9.21
0.10	1.00	1.009	1.09
0.01	0.01	0.011	0.02

From this it becomes manifestly clear that the effect of inbreeding, even when it is considerable, is of little practical significance for the characters which are not rare.

Glass goes further and states that "It is only for loci, such as the MN blood group locus, where dominance is not a complicating factor, that the

allele frequencies can be calculated directly from the phenotypes. It is therefore important to note that if MN phenotypic frequencies in the data of Sanghvi & Khanolkar are converted into the frequencies of $_{L}M$, $_{L}N$, the statistical significance of the differences reported is greatly reduced. χ^2 is reduced by approximately one-half, and this leaves only a single intergroup MN difference significant at the 1% level, instead of three."

TABLE 2

VALUES OF χ^2 FOR INTERGROUP DIFFERENCES WITH RESPECT TO MN BLOOD GROUPS

Group combination	Phenotypes	Genes
V.N.B. × D.B.	11.74***	11.09****
V.N.B. × K.B.	1.88	1.67
V.N.B. × C.K.P.	13.84****	13.58****
V.N.B. × M.K.	9.61***	9.27***
D.B. × K.B.	4.33	3.89*
D.B. × C.K.P.	0.49	0.08
D.B. × M.K.	0.38	0.05
K.B. × C.K.P.	5.73	5.43**
K.B. × M.K.	3.10	2.84
C.K.P. × M.K.	0.41	0.33

Statistical significance: * indicates $.05 > P > .02$
** indicates $.02 > P > .01$
*** indicates $.01 > P > .001$
**** indicates $P < .001$

I fail to understand the types of calculations carried out by Glass to arrive at the conclusions he has made, since my own analysis (Table 2) on a count of M and N genes shows exactly the opposite results. Values of χ^2 have decreased only slightly in every case with a loss of one degree of freedom. Not only has this considerably increased significantly the three intergroup differences but has actually revealed two more significant intergroup differences which were not evident in the earlier analysis.

LITERATURE CITED

Glass, B., 1954, Genetic changes in human populations, especially those due to gene flow and genetic drift. Advances Genet. 6: 95–139.

Haldane, J. B. S. & Koshinsky, P., 1939, Inbreeding in Mendelian populations with special reference to human cousin marriage. Ann. Eugen., 9: 321–340.

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L. D. SANGHVI

HUMAN VARIATION UNIT
INDIAN CANCER RESEARCH CENTER
PAREL, BOMBAY 12, INDIA
March 12, 1955

THE AMERICAN SOCIETY OF NATURALISTS

SECRETARY'S REPORT, 1954

The annual business meeting of the Society was held in Gainesville, Florida on the Campus of the University of Florida in connection with the AIBS on the evening of September 7th, 1954, with President M. Demerec presiding and about sixty members in attendance. A motion was made and unanimously passed to hold the next annual meeting with the AIBS in September, 1955, on the campus of Michigan State College, East Lansing, Michigan. An invitation extended by Dr. W. S. Stone to meet with the Society for the Study of Evolution at the University of Texas, April 7-11, 1955 was accepted.

The report of the Nominating Committee, Viktor Hamburger, chairman, Alan Boyden, and W. R. Singleton was presented by the Secretary. With no further nominations from the floor the following officers were unanimously elected: President for 1955, Dr. Kenneth V. Thimann, Professor of Plant Physiology, Harvard University; Vice President for 1955, Dr. Elmer G. Butler, Professor of Biology, Princeton University.

Individuals, nominated by members of the Society, and approved by the Executive Committee, were elected to membership. A list of those elected in 1954, who have accepted membership, and a supplementary list of those elected in 1953 and not included in the report for that year is appended to this report. Dr. Warder C. Allee was elected to Honorary Membership.

A motion was presented by President Demerec to authorize a separation of the AIBS from the NRC and to empower the Governing Board of the AIBS to make appropriate changes in the constitution to effect this separation and to institute a new class of membership to be known as "individual members." Dr. Bentley Glass, the new Chairman of AIBS, presented reasons for this move and the motion was passed unanimously after some discussion. The action of the Society was in line with that taken by other Member Societies.

A resolution drawn up by Dr. Paul David was presented by Dr. Glass protesting action of the Department of Health, Education and Welfare in denying approval of certain research projects and questioning of certain individuals favorably passed on by scientific advisory councils. This resolution was adopted after discussion. A copy of the resolution submitted to the Department may be secured either from Dr. Glass or the Secretary of the Naturalists.

The Executive Committee recommended that Dr. L. C. Dunn be continued as Editor of the American Naturalist for an unstated period, contingent on his agreement to this action. This recommendation was approved by the members.

The Secretary's Report was read and approved. A vote of thanks and appreciation was extended to Dr. Fred H. Hull, as local representative for

the Gainesville meeting, to Dr. Arnold Grobman and the local committee, to the University of Florida and the AIBS.

At the Gainesville Meeting a Symposium on "The Origin of the Biota of Middle America," arranged by Vice President Chaney, was presented, on Monday afternoon, September 6th. Subjects and speakers were as follows:

The Mexican Flora and its Origin, Aaron J. Sharp
The Ecological Distribution of Vertebrates in a Mexican Cloud Forest, Paul S. Martin
The Mexican Avifauna and its Origin, Pierce Brodkorb
Evolution of Some West Indian Frogs, Coleman J. Goin

To the Secretary and others it seemed that this symposium, presented by four capable and experienced field naturalists, followed closely the stated objective of the Society. Article I, Section 2 reads: "The object of this Society shall be the association of working naturalists for the discussion, advancement and diffusion of knowledge concerning the broader biological problems, including organic evolution, thus serving to correlate the various biological sciences into a common philosophy of biology."

On Tuesday evening, September 7th, Dr. M. Demerec delivered the Presidential Address on the subject "What is a Gene?—Twenty Years Later." Progress in gene study in the past two decades, particularly in the field of bacterial genetics, was ably reviewed. Dr. Bentley Glass presided and introduced the speaker.

At the Berkeley meeting of the AAAS on December 30th, 1954, the Society cosponsored a Symposium on "Modern Views on Tissue Differentiation" arranged by Dr. Adriance S. Foster. Speakers and subjects were as follows:

Differentiation in Apical Meristems, Ernest M. Gifford
Cell Wall Differentiation in Apical Meristems, Flora M. Scott
Histological and Physiological Aspects of Differentiation in Tissue Cultures, Ernest Ball
Experimental Modification of Tissue Patterns in Roots, John G. Torrey

In November Dr. Demerec appointed a Committee, Th. Dobzhansky, Chairman, John Moore, and Bruce Wallace, to study the matter of clarification of By-law 3 of the Constitution in consultation with Dr. L. C. Dunn. This by-law is at present ambiguous in that it neither states the number of members to be appointed to the Editorial Board of the American Naturalist nor the length of the term of service. This Committee has met and drafted a clarifying revision of this by-law which will be presented at the next annual meeting.

The Executive Committee of the Naturalists for the year 1955 consists of K. V. Thimann, Chairman, E. G. Butler, R. W. Chaney, M. Demerec, W. P. Spencer, C. P. Swanson, and Sewall Wright. Representatives on the AAAS Council for 1955 are H. H. Plough and S. G. Stephens, and Representative on the AIBS Governing Board for 1955 is C. G. Huff.

During the past year the following deaths occurred: L. H. Bailey, A. F. Blakeslee, J. H. Bodine, William Coker, E. A. Hooton, Herbert Osborn, G. H. Shull, and L. J. Stadler. The year 1954 closed with an active membership of 468. It should be understood that active members, on retirement

from their professional posts, may become emeritus members, with exemption from dues. Emeritus members may continue to subscribe to the American Naturalist for the $3.50 subscription rate paid by the Society for each active member. Request for emeritus status should be sent to the Secretary.

In conclusion the Secretary asks your continued cooperation in making nominations to membership of individuals who are making significant contributions to Biology in any of the varied fields.

Respectfully submitted,
WARREN P. SPENCER
Secretary

DEPARTMENT OF ZOOLOGY
UNIVERSITY OF TEXAS
AUSTIN 12, TEXAS

NEW MEMBERS, 1954

R. W. Allard, University of California, Davis
L. Joe Berry, Bryn Mawr College
J. Wallace Boyes, McGill University
Walter J. Burdette, Louisiana State University
Adriano A. Buzzati-Traverso, Scripps Institution of Oceanography
A. F. Carr, University of Florida
Robert T. Clausen, Cornell University
A. Orville Dahl, University of Minnesota
H. Clark Dalton, Washington Square College
H. L. Eastlick, State College of Washington
Martin Gibbs, Brookhaven National Laboratory
Verne Grant, Rancho Santa Ana Botanic Garden, Cal.
Allen B. Griffen, Roscoe B. Jackson Memorial Laboratory, Bar Harbor
Melville H. Hatch, University of Washington
W. E. Heston, National Cancer Institute, Bethesda
Horton H. Hobbs, University of Virginia
Johannes Holtfreter, University of Rochester
Walter E. Howard, University of California, Davis
R. E. Hungate, State College of Washington
James C. King, Biological Laboratory, Cold Spring Harbor
I. L. Kosin, State College of Washington
Harry H. Laidlaw, University of California, Davis
W. Harry Lange, University of California, Davis
Howard Levene, Columbia University
R. Merton Love, University of California, Davis
George H. Mickey, Northwestern University
Ray Moree, State College of Washington
Emil M. Mrak, University of California, Davis
Leslie F. Nims, Brookhaven National Laboratory
Eugene P. Odum, University of Georgia
H. P. Olmo, University of California, Davis
Marion Ownbey, State College of Washington
Herman J. Phaff, University of California, Davis
S. R. M. Reynolds, Carnegie Institution of Washington
Herschel Roman, University of Washington

Sherman Ross, University of Maryland
K. H. Rothfels, University of Toronto
Charles H. Seevers, Roosevelt University, Chicago
Ernest H. Stanford, University of California, Davis
J. P. Trinkaus, Yale University

NEW MEMBERS, 1953—SUPPLEMENTARY TO LIST IN MARCH-APRIL, 1954—THE AMERICAN NATURALIST

J. B. Birdsell, University of California, Los Angeles
Frank Brink, Jr., Johns Hopkins University
Ralph O. Erickson, University of Pennsylvania
Stanley M. Garn, Fels Research Institute, Antioch College
H. K. Hartline, Johns Hopkins University
Rollin D. Hotchkiss, Rockefeller Institute for Medical Research
W. W. Howells, University of Wisconsin
David R. Lincicome, The Stine Laboratory, E. I. Du Pont, Newark, N. J.
Walter J. Nickerson, Rutgers University
Edward Novitski, University of Missouri
J. N. Spuhler, University of Michigan
Leo Szilard, Brandeis University
Evelyn M. Witkin, Carnegie Inst. of Washington, Cold Spring Harbor

REPORT OF THE EDITOR, 1954-5

Almost twice as many manuscripts were received during 1954 as in the preceding year. A total of 88 manuscripts were considered. Of these 48 were published, 16 rejected, and 24 held over for 1955. Two long papers were published as supplements, the extra costs being paid for by the authors, as was the additional material added to the July issue which contained the Symposium on Some Biological Effects of Radiation from Nuclear Detonations. The result was a considerable expansion of Volume 88 for 1954.

There was as usual a wide variation in the subject matter of the papers submitted, with genetics, especially mutation, leading the list. The large amount of material submitted will now permit us to adhere more closely to the editorial policy announced in January, 1951, by which a balance representing the various fields of interest of the Society may be maintained by giving preference to papers of general biological interest "in which theoretical interpretation and synthesis are predominant." In pursuit of the above purpose the Board also decided to invite reviews of new books of wide interest in which a reviewer is able to summarize progress and changes of view in some general field.

The editor wishes to record his gratitude to the members of the Board and to Mr. Marvin L. Edwards, editorial assistant, in carrying the chief burden of editorial work during his residence in Europe during 1953-4.

L. C. DUNN

REPORT OF TREASURER

November 16, 1954

AMERICAN SOCIETY OF NATURALISTS

Balance: received from D. F. Poulson, January 1954		$1,330.95
Income: from dues: January 15–November 16, 1954		2,854.50
Income: other sources: Dividend check, Woodlawn Trust and Savings Bank, Chicago		6.06
Total Receipts		$4,191.51
Expenditures: January–November 1954 Science Press—subscriptions for American Naturalist	$1,764.00	
Science Press—printing of Records	712.36	
L. C. Dunn, editorial assistance	300.00	
W. P. Spencer, Sec'y, travel, Boston, Dec. 1953	89.46	
W. P. Spencer, Sec'y, travel, Gainesville, Sept. 1954	105.80	
Express charges, shipping records from New Haven to Baltimore	2.65	
Union News Pub. Co., printing dues cards	22.95	
JHU Bookstore, rubber stamp and account journal	3.91	
National Academy of Sciences, AIBS membership	251.00	
Total Expenditures		$3,252.13
Balance, First National Bank, Baltimore, November 16, 1954		$ 939.38

C. P. SWANSON
Treasurer

REPORT OF SPECIAL COMMITTEE ON THE AMERICAN NATURALIST

Your committee (Dobzhansky, Moore, Wallace) met on December 15, 1954, in consultation with Professor L. C. Dunn, Editor of *The American Naturalist.*

We recommend that the By-law 3 of the Constitution of the Society be modified to read as follows:

"3. The Executive Committee shall be empowered to appoint an Editor for *The American Naturalist*, to serve for a five year term. The Executive Committee, in consultation with the Editor, shall appoint an Editorial Board to advise the Editor on matters of policy. Each member of the Editorial Board shall serve for a term of three years."

As a temporary measure for the transition period between the present and the time when the modified by-law will take its full effect, we recommend to the Executive Committee that the members of the Editorial Board be divided into three classes as follows:

Class 1955 (to serve until December 31, 1955)
Marston Bates
D. D. Keck
Carl L. Hubbs
Libbie H. Hyman (new appointment)

Class 1956 (to serve until December 31, 1956)
A. S. Foster
Daniel Mazia (new appointment)
Jack Schultz
J. Ledyard Stebbins, Jr.

Class 1957 (to serve until December 31, 1957)
T. Dobzhansky
G. Evelyn Hutchinson
Thomas Park
Conway Zirkle

All the recommendations were accepted by the committee unanimously except the suggestion of Dobzhansky as a member of the Editorial Board, which was accepted by a 2 to 1 majority. With the arrangement as proposed, the term of Professor L. C. Dunn as Editor will expire at the end of 1955.

December 15/1954 Th. Dobzhansky

The report of this committee has been accepted by the Executive Committee.

PUBLICATIONS RECEIVED

THE AMERICAN NATURALIST is glad to acknowledge here the receipt of books on biological and natural history subjects which are likely to be of interest to our readers. No undertaking to publish reviews is implied in this acknowledgment. Books for notice may be sent to:

EDITORIAL OFFICE, The American Naturalist
Box 2, Schermerhorn Hall, Columbia University
New York 27, N. Y.

L'Heritier, Ph., 1954. Traité de génétique. 2 vols.: Vol. 1, Le mecanisme de l'hérédité genetique formella. Vol. 2, La génétique des populations. 518 p., ill. 1500 fr. + 900 fr. Presses Universitaires de France, Paris, France.

A general elementary treatise to be completed with volume 3—Génétique physiologique (in preparation).

Mann, Guillermo, 1954. La vida de los peces en aguas chilenas. 342 p., ill. Ministerio de Agricultura, Santiago de Chile.

The second edition of this key to fishes found in the waters of Chile has been expanded to include not only the teleost fishes (as in the first edition of 1950) but all fishes, giving common and specific names.

Mohr, Jan, 1954. A study of linkage in man. 119 pp. Einar Munksgaard, Copenhagen.

A review of human linkage data published before 1953, and a report of a search for linkage in new family material, presented in detail, between marker genes (chiefly blood group factors) and other hereditary conditions. One linkage between Lewis and Lutheran blood group genes is disclosed with crossover value estimated at eight percent.

Neel, James V., and William J. Shull, 1954. Human heredity. 361 p., ill. $6.00. University of Chicago Press, Chicago.

An up-to-date discussion of the problems of human genetics, in the form of a text-book for students or those who have studied general genetics, with especial emphasis on methods (probability, estimation of genetic parameters, analysis of family data).

Noailles, R. H. and Guicher, J. M., 1954. The hidden life of flowers. 93 pp., 103 photogravure illustrations. $4.75. The Philosophical Library, N. Y.

A book of beautiful photographs of flowers and floral parts mady by a French photographer, beautifully reproduced by British engravers and printers, built around the theme of the birth and development of a plant, integrated by the sensitive and accurate commentary of J. M. Guicher,

the whole rendered into clear and poetic English. It is a book for old and young, and might make more botanists than a text book.

Sheldon, William H., 1954. Atlas of men, a guide and handbook on somatotyping for those concerned with problems of human physique. 357 p., 1175 somatotype photographs, 19 tables, 5 figures. $10.00. Harper & Brothers, New York.

A complete description and arrangement of the 88 categories of physique (somatotypes) found in the American male population, together with age, height and weight standards for each category.

Simpson, George, 1954. Man in society. Preface to sociology and the social sciences. 90 pp. $.95, paper. Doubleday Short Studies in Sociology. Doubleday & Co., Inc., Garden City.

Wagner, Florence Signaigo, 1954. Contributions to the morphology of the Delesseriaceae. Vol. 27, No. 5, pp. 279-346 in the University of California publications in botany. 290 text-figures. $1.00. University of California Press, Berkeley, California.

Wenrich, D. H., Ivey F. Lewis, and John R. Raper, editors, 1954. Sex in microorganisms. 362 p., ill. $5.75 ($5.00 to AAAS members). American Association for the Advancement of Science, Washington, D. C.

This volume comprises papers given at a A.A.A.S. Symposium at Philadelphia on December 30, 1951. There may be some moral to be drawn from noting that it appeared contemporaneously with a volume on *Autotrophic Microorganisms* which was published by our British colleagues and which embodied a symposium held in London, April 1954! Yet, because most of the articles contained refer to papers published as recently as 1953, the lapse of time between the Symposium and publication has not caused the volume to be more out of date than is usual for reviews in fields expanding as rapidly in data and ideas as is the genetics of microorganisms. The component papers deal with various aspects of "sex" in viruses, bacteria, algae, protozoa and fungi and the mode of treatment is very uneven. For example, knowledge of sexual systems in both protozoa and fungi is extensive and diverse; Raper's treatment of the fungi is formal and synthetic while Wenrich's discussion of the protozoa is specific and detailed (and includes a useful summary of Cleveland's remarkable work on animal flagellates). Some of the papers are more restricted in scope; Visconti clearly and very succinctly reviews recombination in bacterial viruses while Nanney deals with the determination of mating type in *Paramecium aurelia* in a more analytical fashion. Nonetheless, *all* of the papers make interesting reading and the volume will serve as a source book in its field.

Wilson, William, 1954. The microphysical world. 216 p. $3.75. Philosophical Library, New York.